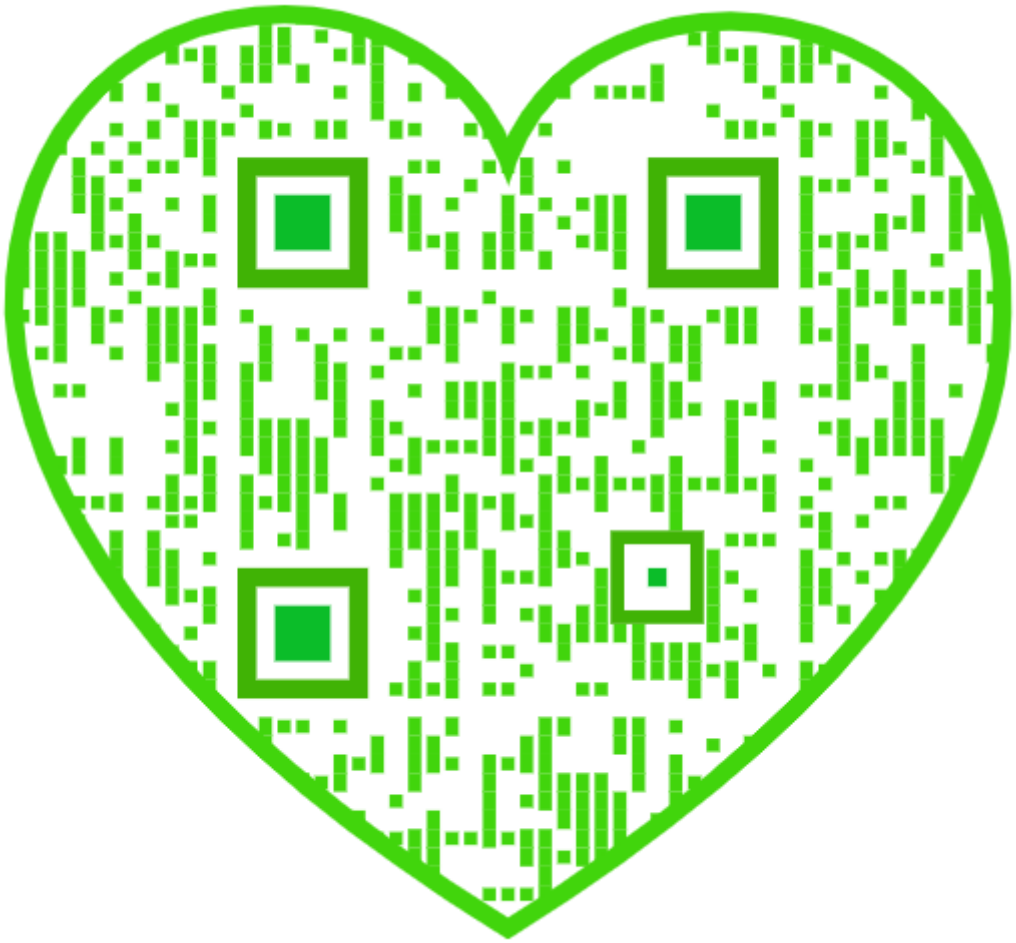
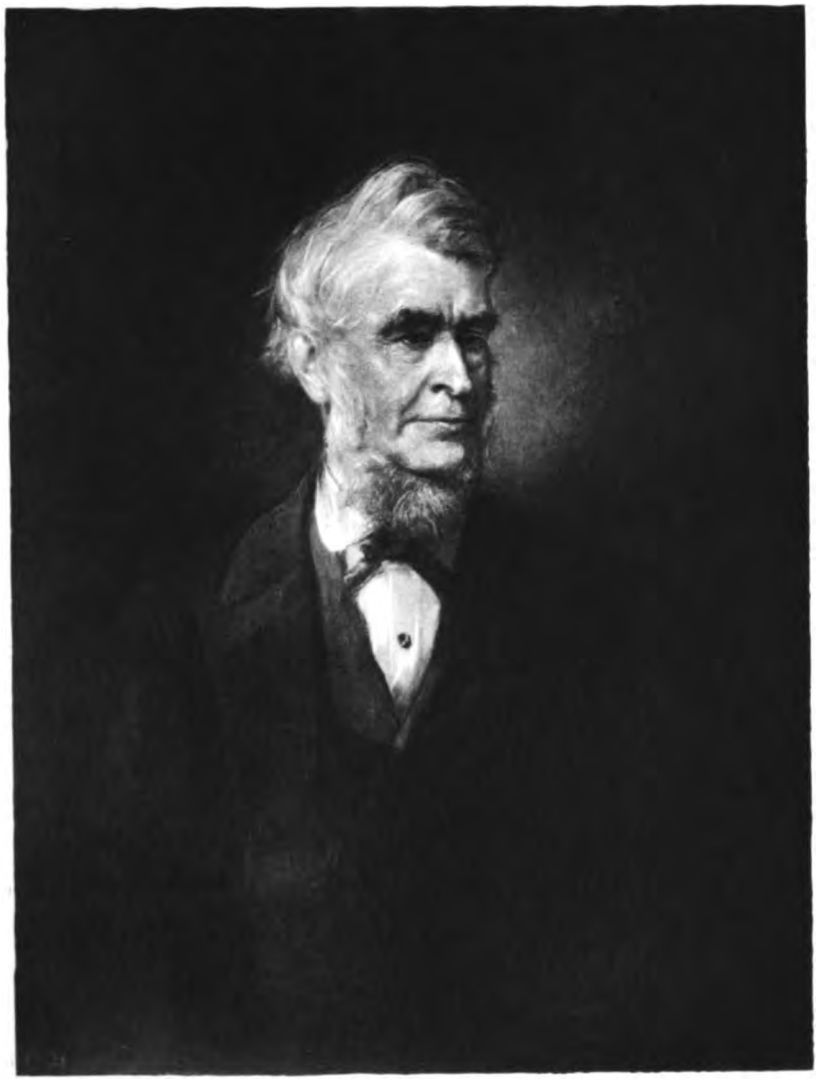


4//34

ANNALS OF BOTANY

VOL. XII



George Bentham

ANNALS OF BOTANY

EDITED BY

ISAAC BAYLEY BALFOUR, M.A., M.D., F.R.S.

QUEEN'S BOTANIST IN SCOTLAND, PROFESSOR OF BOTANY IN THE UNIVERSITY AND KEEPER OF THE ROYAL BOTANIC GARDEN, EDINBURGH

SYDNEY HOWARD VINES, M.A., D.Sc., F.R.S.

FELLOW OF MAGDALEN COLLEGE, AND SHERARDIAN PROFESSOR OF BOTANY IN THE UNIVERSITY OF OXFORD

D. H. SCOTT, M.A., PH.D., F.R.S.

HONORARY KEEPER OF THE JODRELL LABORATORY, ROYAL GARDENS, KEW

AND

WILLIAM GILSON FARLOW, M.D.

PROFESSOR OF CRYPTOGAMIC BOTANY IN HARVARD UNIVERSITY, CAMBRIDGE, MASS., U.S.A.

ASSISTED BY OTHER BOTANISTS

VOLUME XII

London

HENRY FROWDE, M.A., AMEN CORNER, E.C.

OXFORD: CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1898

LIBRARY OF THE
LELAND STANFORD JR. UNIVERSITY.

a.43876.

SEP 4 1900

Oxford
PRINTED AT THE CLARENDON PRESS
BY HORACE HART, M.A.
PRINTER TO THE UNIVERSITY

CONTENTS.

No. XLV, March, 1898.

PAGE

CAMPBELL, D. H.—The Development of the Flower and Embryo in Lilaea subulata, H. B. K. (With Plates I–III) . . . 1

WEST, W. and WEST, G. S.—Observations on the Conjugatae. (With Plates IV and V) 29

WARD, H. M.—A Violet Bacillus from the Thames. (With Plate VI) 59

CHURCH, A. H.—The Polymorphy of Cutleria multifida, Grev. (With Plates VII–IX) 75

DAWSON, M.—On the Structure of an Ancient Paper 111

NOTES.

TOWNSEND, C. O.—Correlation of Growth under the Influence of Injuries 117

DIXON, H. H.—Gelatine as a Fixative 117

GROOM, P.—Lathraea Squamaria 118

No. XLVI, June, 1898.

JOHNSON, D. S.—On the Development of the Leaf and Sporocarp in Marsilia quadrifolia, L. (With Plates X–XII) . . . 119

PARKIN, J.—On some points in the Histology of Monocotyledons. (With Plate XIII) 147

MAGNUS, P.—On Aecidium graveolens (Shuttlew.). (With Plate XIV) 155

BIFFEN, R. H.—The Coagulation of Latex 165

PHILLIPS, R. W.—The Development of the Cystocarp in Rhodymeniales: II. Delesseriaceae. (With Plates XV and XVI) . 173

WORSDELL, W. C.—The Vascular Structure of the Sporophylls of the Cycadaceae. (With Plates XVII and XVIII) . . . 203

REID, C.—Further Contributions to the Geological History of the British Flora. 243

NOTES.

LANG, W. H.—On Apogamy and the Development of Sporangia upon Fern-Prothalli 251

MASLEN, A. J.—The Ligule in Lepidostrobus. (With Woodcut 1) . 256

No. XLVII, September, 1898.

PAGE

SHAW, W. R.—The Fertilization of Onoclea. (With Plate XIX) . 261
WARD, H. M.—Some Thames Bacteria. (With Plates XX and XXI) 287
HILL, T. G.—On the Roots of Bignonia. (With Plate XXII) . . 323
BARBER, C. A.—Cupressinoxylon vectense. (With Plates XXIII and XXIV) 329
EWART, A. J.—The Action of Cold and of Sunlight upon Aquatic Plants 363
SCOTT, R., and SARGANT, E.—On the Development of Arum maculatum from the Seed. (With Plate XXV) 399

NOTES.

EWART, A. J.—The Action of Chloroform on CO_2-assimilation . . 415
LEWIS, F. J.—The Action of Light on Mesocarpus 418

No. XLVIII, December, 1898.

GANONG, W. F.—Contributions to a Knowledge of the Cactaceae: II. The Comparative Morphology of the Embryos and Seedlings. (With Plate XXVI) 423
PEARSON, H. H. W.—Anatomy of the Seedling of Bowenia spectabilis. (With Plates XXVII and XXVIII) 475
GREEN, J. R.—The Alcohol-producing Enzyme of Yeast . . . 491
WAGER, H.—The Nucleus of the Yeast-Plant (With Plates XXIX and XXX) 499
VINES, S. H.—The Proteolytic Enzyme of Nepenthes, II . . . 545

NOTES.

BURKILL, I. H.—Changes in the Sex of Willows 557
JONES, C. E.—Anatomy of the Stem of Species of Lycopodium . . 558
WILLIAMS, J. LLOYD.—Reproduction in Dictyota dichotoma . . 559
HUIE, L. H.—Changes in the Gland-Cells of Drosera produced by various Food-materials 560
WARD, H. M.—A Potato-Disease 561
Penicillium as a Wood-destroying Fungus 565
ELLIS, W. G. P.—A Method of obtaining Material for illustrating Smut in Barley 566
ERRERA, L.—Structure of the Yeast-Cell 567
Osmotic Optimum and Measurements 568
PHILLIPS, R. W.—The Form of the Protoplasmic Body in certain Florideae 569
KLEBS, G.—Alternation of Generations in the Thallophytes . . 570
LANG, W. H.—Alternation of Generations in the Archegoniatae . . 583
HARTOG, M.—Alternation of Generations 593

CONTENTS AND INDEX . . . . . i–viii
HOOKER, SIR J. D.—Biographical Memoir of George Bentham. (With Portrait) . . . . . ix–xxx

INDEX.

A. ORIGINAL PAPERS AND NOTES.

PAGE

BARBER, C. A.—Cupressinoxylon vectense. (With Plates XXIII and XXIV) 329
BIFFEN, R. H.—The Coagulation of Latex 165
BURKILL, I. H.—Changes in the Sex of Willows 557
CAMPBELL, D. H.—The Development of the Flower and Embryo in Lilaea subulata, H. B. K. (With Plates I–III) 1
CHURCH, A. H.—The Polymorphy of Cutleria multifida, Grev. (With Plates VII–IX) 75
DAWSON, M.—On the Structure of an Ancient Paper 111
DIXON, H. H.—Gelatine as a Fixative 117
ELLIS, W. G. P.—A Method of obtaining Material for illustrating Smut in Barley 566
ERRERA, L.—Structure of the Yeast-Cell 567
Osmotic Optimum and Measurements 568
EWART, A. J.—The Action of Cold and of Sunlight upon Aquatic Plants 363
The Action of Chloroform on CO_2-assimilation 415
GANONG, W. F.—Contributions to a Knowledge of the Cactaceae: II. The Comparative Morphology of the Embryos and Seedlings. (With Plate XXVI) 423
GREEN, J. R.—The Alcohol-producing Enzyme of Yeast 491
GROOM, P.—Lathraea Squamaria 118
HARTOG, M.—Alternation of Generations 593
HILL, T. G.—On the Roots of Bignonia. (With Plate XXII) . . . 323
HOOKER, J. D.—Biographical Memoir of George Bentham. (With Portrait) ix
HUIE, L. H.—Changes in the Gland-Cells of Drosera produced by various Food-materials 560
JOHNSON, D. S.—On the Development of the Leaf and Sporocarp in Marsilia quadrifolia, L. (With Plates X–XII) 119
JONES, C. E.—Anatomy of the Stem of Species of Lycopodium . . 558
KLEBS, G.—Alternation of Generations in the Thallophytes . . 570
LANG, W. H.—On Apogamy and the Development of Sporangia upon Fern-Prothalli 251
Alternation of Generations in the Archegoniatae 583
LEWIS, F. J.—The Action of Light on Mesocarpus 418
MAGNUS, P.—On Aecidium graveolens (Shuttlew.). (With Plate XIV) 155
MASLEN, A. J.—The Ligule in Lepidostrobus. (With Woodcut 1) . 256

PAGE

PARKIN, J.—On some points in the Histology of Monocotyledons. (With Plate XIII) 147
PEARSON, H. H. W.—Anatomy of the Seedling of Bowenia spectabilis. (With Plates XXVII and XXVIII) 475
PHILLIPS, R. W.—The Development of the Cystocarp in Rhodymeniales: II. Delesseriaceae. (With Plates XV and XVI) . 173
The Form of the Protoplasmic Body in certain Florideae . . 569
REID, C.—Further Contributions to the Geological History of the British Flora 243
SCOTT, R., and SARGANT, E.—On the Development of Arum maculatum from the Seed. (With Plate XXV) 399
SHAW, W. R.—The Fertilization of Onoclea. (With Plate XIX) . 261
TOWNSEND, C. O.—Correlation of Growth under the Influence of Injuries 117
VINES, S. H.—The Proteolytic Enzyme of Nepenthes, II . . . 545
WAGER, H.—The Nucleus of the Yeast-Plant. (With Plates XXIX and XXX) 499
WARD, H. M.—A Violet Bacillus from the Thames. (With Plate VI). 59
Some Thames Bacteria. (With Plates XX and XXI) . . . 287
A Potato-Disease 561
Penicillium as a Wood-destroying Fungus 565
WEST, W., and WEST, G. S.—Observations on the Conjugatae. (With Plates IV and V) 29
WILLIAMS, J. LLOYD.—Reproduction in Dictyota dichotoma . . 559
WORSDELL, W. C.—The Vascular Structure of the Sporophylls of the Cycadaceae. (With Plates XVII and XVIII) . . . 203

B. LIST OF ILLUSTRATIONS.

a. PLATES.

Portrait of George Bentham (Frontispiece).
I, II, III. Development of Flower and Embryo in Lilaea subulata (CAMPBELL).
IV, V. Observations on Conjugatae (WEST, W., and WEST, G. S.).
VI. Violet Bacillus from the Thames (WARD).
VII, VIII, IX. Polymorphy of Cutleria multifida (CHURCH).
X, XI, XII. Development of Leaf and Sporocarp in Marsilia quadrifolia (JOHNSON).
XIII. Some points in the Histology of Monocotyledons (PARKIN).
XIV. Aecidium graveolens (MAGNUS).
XV, XVI. Development of Cystocarp in Delesseriaceae (PHILLIPS).
XVII, XVIII. Vascular Structure of Sporophylls of Cycadaceae (WORSDELL).
XIX. Fertilization of Onoclea (SHAW).
XX, XXI. Some Thames Bacteria (WARD).
XXII. Roots of Bignonia (HILL).
XXIII, XXIV. Cupressinoxylon vectense (BARBER).
XXV. Development of Arum maculatum (SCOTT and SARGANT).
XXVI. Comparative Morphology of Embryos and Seedlings of Cactaceae (GANONG).
XXVII, XXVIII. Anatomy of Seedling of Bowenia spectabilis (PEARSON).
XXIX, XXX. Nucleus of the Yeast-Plant (WAGER).

b. WOODCUT.

1. Ligule in Lepidostrobus (MASLEN).

GEORGE BENTHAM, F.R.S.

(*With Portrait.*)

THE following account of the life and labours of George Bentham is based on an obituary notice which I communicated to Nature (Vol. XXX, October 1884, p. 359). In reproducing it in the present form, I have enlarged it considerably, and further availed myself of four subsequent accounts, namely, of Mr. Thiselton-Dyer's Eulogium, read before the Linnean Society (Proceedings, Sessions 1887–1889); of Prof. Gray's Memorial, presented to the American Academy of Arts and Sciences (Journal, Vol. XXIX, February, 1885); of Prof. Oliver's Obituary notice (Proc. Royal Society, 1885), and of Mr. Daydon Jackson's notice (Proc. Linn. Soc., Session 1884–5). The reminiscences of his very early life are taken from an autobiography which he commenced very shortly before his death, but which he was unable to continue.

The life of George Bentham presents such variety, such startling changes of conditions, and a combination of so many natural and acquired mental powers of a high order, that it cannot be perused without the question arising, how far heredity and environments had influenced his career. Such being the case, I think no apology is needed for commencing this sketch with some account of his parentage.

He was born on September 22, 1800, in the village of Stoke, near Portsmouth. His father, afterwards Sir Samuel Bentham, who was the son of a wealthy scrivener in the Minories, and the only brother of Jeremy Bentham the publicist, devoted himself as a youth to the study of Naval Architecture, and at the age of 22, at the suggestion of Lord Howe, went to Russia with the view of further instructing

himself in that science. Then he travelled to the Crimea, visited the naval establishments in the Baltic and the Black Sea, and thence went on to Siberia (penetrating to the frontiers of China) for the purpose of making himself acquainted with the mines, foundries, and other great industries of that country. Meanwhile he had gained the friendship of Prince Potemkin, who, impressed by his genius and ability, induced him to enter the service of the Empress Catherine II, who gave him a Lieutenant-Colonel's commission, without requiring him to pass through the subaltern grades of the army. In this capacity he was sent to the Crimea, where, amongst many other engineering feats, he built a flotilla of gun-boats, in command of which (under Prince Potemkin) he gained a signal victory over the whole Turkish fleet in the Black Sea. For this he received from the Empress the cross of St. George, conferring Knighthood, a sword of honour, and promotion to the rank of Colonel in command of a cavalry regiment in Siberia, which country he re-traversed from the Obi to the Amur, engaged chiefly in the construction of boats for the navigation of the Siberian rivers.

After the death of the Empress he returned to England, left the Russian service, and entered that of the Admiralty, by whom he was commissioned to return to Russia, and there superintend the building of some ships for the British Navy. Thither he went with his wife and family, including George, and remained, till the declaration of war with that country required his recall. Finally he rose to be Inspector of all our dockyards, in which capacity he introduced a multitude of improvements, including steam saw- and other mills, the replacement of water-casks in ships by iron tanks; and with Sir Isambard Mark Brunel, whom he brought over from the Continent, he constructed the eccentric machinery for turning elliptic blocks[1].

G. Bentham's mother was the daughter of Dr. G. Fordyce, F.R.S., an eminent London physician, and lecturer on chemistry, author of various works on medicine and agri-

[1] Sir Samuel Bentham's portrait hangs in Greenwich Hospital.

culture. She was a woman of remarkable power of mind, who aided her father in his scientific labours, and her husband in preparing his voluminous official reports to the Admiralty. At the age of 80 she wrote a beautiful hand, and during the Crimean war, when considerably over 90, she commenced a series of letters to the *Times*, urging the adoption of guns of a large calibre, and other improvements in war-material, the inventions of her late husband, whose Life she published in 1862.

Not less influential on George Bentham's career was the teaching of his uncle Jeremy, who imbued him with that love for methodical and logical analysis which is so conspicuous in all his nephew's writings. As has been well remarked in this relation, 'The same inherited aptitude and contemporary influences which produced a great publicist in Jeremy, yielded, by an almost accidental deflection, a great systematic botanist in his nephew' (Eulogium, p. 8).

Environments were as favourable to Bentham in his scientific career, as were the qualities of his progenitors. He was one of five children (three of them girls), all of them precocious. They were taught to read by words, not by syllables or letters, and the two brothers commenced learning Latin before they were five years old. In 1805 the whole family accompanied the father to Russia, where their education was entrusted to a talented Russian lady who could speak no English, whilst in Latin the boys were instructed by a Russian priest, of whom George in after life always spoke with great regard. Music, of which the latter became passionately fond, was not neglected, and it resulted in his becoming an accomplished pianist. Thus, having a remarkable facility for acquiring languages, Bentham could, at seven years old, converse fluently in English, French, German, and Russian, to which, by hard work, he added Swedish, during a detention of some weeks at Carlscrona on the voyage back to England. The said voyage proved a tempestuous and perilous one. Embarking at Revel in a Russian frigate, with a crew, few of whom had ever before seen the sea, they were tossed about in the Baltic

for five weeks before arriving at Carlscrona; and there were as many equally stormy weeks in their passage thence in an ill-found merchantman to Harwich, where the family arrived in a half-starved condition, having been reduced to picking up stray scraps of biscuit in out-of-the-way corners of the cabins.

In England Sir Samuel Bentham took up his residence at Hampstead in the summer months, daily visiting his office at the Admiralty. In winter he resorted to a small house and property called Berry Lodge[1], between Alverstoke and Gosport, which was in convenient proximity to Portsmouth Dockyard. The boys meanwhile pursued their studies under private tutors, a plan continued throughout the whole course of George's education. It was a life-long source of regret with George that he had never been sent to school or college, which may account for a shyness and reserve, attributed by those that did not know him to a want of sympathy.

In 1812–13, the invasion of Russia by Napoleon, and the burning of Moscow, naturally caused great excitement in the Bentham family. It led to the first appearance of George, then only 13, before the public; he, with his brother and sisters, contributing to the London Magazine a series of papers, gleaned from Russian sources, detailing the operations of the armies, and glorying in the reverses and final abdication of Napoleon.

After the proclamation of peace, Sir Samuel Bentham took his family to France, and resided successively at Tours, Saumur, and Paris. During this period, which extended from Napoleon's return from Russia to his final overthrow, young Bentham kept a full journal of all that passed, interspersed with anecdotes relating to the forced exile of Louis, the restoration of the Bourbons, the execution of Ney and

[1] It was from here that, while George was still in his teens, his father took him on a visit to Lady Spencer at Ryde, at whose house he met John Stuart Mill, a lad of six, dressed in a scarlet jacket buttoned over nankeen trousers, and considered to be a prodigy. Bentham has described him to me as having been wonderfully precocious, a Greek and Latin scholar, historian and logician, whom he heard discussing with Lady Spencer the relative merits of her ancestor, the Duke of Marlborough, and of Wellington, young Mill taking the part of the latter.

Labedoyère, the condition of the city of Paris, and to Walter Savage Landor, who was intimate with the family. Even at this age he could take his part in the society of the leaders of the Paris *Salons* in literature and science, making the acquaintance of the Duc de Richelieu, Talleyrand, Dumas, Jean Baptiste Say, the aged Madame Andelau (daughter of Helvetius), and Alexander Humboldt. Of these the latter took an especial interest in him, encouraging him in the prosecution of a work he had begun on the data of physical geography, by advice and by procuring him introductions to libraries and to individuals who could aid him. Unfortunately this projected work was not continued.

In 1816 Sir Samuel Bentham organized at Paris a caravan-tour in France for himself and family. The caravan consisted of a two-horse coach fitted up as a sleeping-room, a one-horse spring van furnished with a library and piano, for himself and Mrs. Bentham, and another for his daughters and their governess. Thus equipped they travelled by day, visiting friends and places of interest, bivouacking by night in gipsy fashion in the gardens of friends, or in the precincts of the prefectures, to which he brought credentials from Paris. In this way he visited Orléans, Tours, Angoulême, Bordeaux, Toulouse, Montpellier, and finally Montauban, where the caravan having broken down, the tour was continued by ordinary conveyances to Carcassonne, Narbonne, Nîmes, Tarascon, Marseilles, Toulon, and Hyères.

The most interesting incident of this tour occurred at Angoulême, for there G. Bentham's attention was first directed to botany. His mother, who was fond of plants, and a friend of Aiton of Kew, had purchased a copy of De Candolle's just then issued 'Flore Française.' Young Bentham accidentally taking it up was interested in the analytical tables for determining the affinities and names of the plants described, which fitted in with the ideas he had derived from his uncle Jeremy's works, when constructing his own geographical tables. He at once went into the yard of the house, gathered the first plant he found, and after spending

the morning in studying its structure, with the aid of the introductory chapter of the 'Flore,' succeeded in referring it to its order, genus, and species. The plant, *Salvia pratensis*, is not an easy one for a beginner. Encouraged by his success with it, he pursued the study of the native flora as a diversion, naming every plant he subsequently met with.

At Montauban, where his father had purchased a country house, which the family occupied for about two years, young George Bentham passed what he always regarded as the most enjoyable period of his life. He was entered as a student in a Protestant theological college, and followed with ardour the courses of mathematics, Hebrew, and comparative philology, the latter a favourite study in after life. At home, during the holidays, he occupied his time with drawing plants, learning Spanish, and with music, society, and dancing, of which latter he was passionately fond. It was a favourite boast of his, that at Montauban he had danced at thirty-four balls between Twelfth-night and Mardi-gras, of which thirteen were consecutive, and lasted from 9 p.m. to 9 a.m. Here, too, his mind was first opened to scientific and exotic botany, to which he was led by the works of De Candolle, by the appearance of the 'Dictionnaire d'Histoire Naturelle,' and by a course of lectures under Benedict Prevost. This was followed by a devotion to ornithology, including shooting and stuffing birds, and that again by entomology, tabulating the phenomena of insect-life. Here, too, probably inspired by John Stuart Mill, who resided for some time with the family, his mind was turned to philosophy and the study of Lamarck's works, beginning with the 'Système analytique des Connaissances positives de l'homme,' only to give it up with disgust on reading that 'Dieu créa d'abord la matière,' followed by the statement that Nature was the second thing created, and that this produced everything else. More to the purpose was his translation into French of his uncle's Chrestomathia, which was a prelude to his becoming secretary to the great publicist at a later period.

From Montauban Sir Samuel Bentham moved to a large

estate of 2,000 acres, which he had purchased, near Montpellier, and the management of which he made over to his now only son, the eldest having died some years previously. The estate consisted of farms and vineyards, to the improvement of which George devoted himself with alacrity and success. They became very profitable, and throughout the remainder of his life in England an excellent St. George Burgundy (the produce of the Restinalières estate), and a rare and luscious Lunel from a neighbouring vineyard, were familiar to the guests at his table. All his spare time was devoted to botanical excursions in the Cevennes and Pyrenees, and to making a French translation of his uncle's Essay on Nomenclature and Classification. Here, too, he wrote his first important work, 'Essai sur la Nomenclature et Classification des Arts et Sciences,' which was published in Paris, and which established his position as an acute analyzer, clear expositor, and cautious reasoner. Half a century after its appearance it was praised by Professor Stanley Jevons in his History of the Sciences.

In 1823 G. Bentham was sent to England for the purpose of obtaining agricultural implements and information as to improved methods of farming. On his arrival in London he was well received by his uncle, and introduced into the best literary and scientific society of the capital. He was invited to the breakfasts and receptions of Sir Joseph Banks, and studied in his library and herbarium, where he commenced a life-long friendship with their curator, Robert Brown, 'Botanicorum facile princeps.' There, and at the Horticultural and Linnean Societies, he met the élite of the naturalists of the day. From London he made a tour into Scotland, where he was hospitably entertained by the Professors of Botany in Edinburgh (Graham) and Glasgow (Hooker)[1], and

[1] It was from this visit that Bentham was wont to date his permanent adherence to Botany. I, then six years old, remember him and the enthusiasm with which he received from my father a collection of Alpine Scotch plants, the first examples of the Northern European Flora he had ever seen. The intimate friendship between my father and Bentham, which lasted forty-two years, dates from this period.

by Arnott, of Arlary, in Kinross-shire, who was subsequently Professor of Botany in Glasgow. With the latter he arranged to make an extended botanical excursion in the Pyrenees, which was carried out in 1824, and which resulted in his first botanical work, 'Catalogue des Plantes Indigènes des Pyrénées et du Bas Languedoc, avec des notes et observations' (Paris, 1826). Another result of his Pyrenean exploration was the publication in the London Magazine for 1827 of two articles entitled 'Sketches of Manners in the South of France,' wherein, amongst much curious philological and other matter relating to the Roussillonais, Catalonians, and Languedociens, an account is given of a visit to the Lilliputian Republic of Andorra, its physical features, people, government, agriculture, and productions. These sketches are masterpieces of their kind.

In 1826 the Restinalières estate had to be abandoned, owing to provincial jealousy, which threw every obstacle in the way of improvements, and the Bentham family returned to England for good. Here a new, and as it proved, a very uncongenial career was opened to George. His uncle Jeremy, gratified by the translation of Chrestomathia, invited his nephew to be his aid in arranging his MSS. for the press, accompanying it with the assurance that he would provide for him at his death. This invitation was accepted, but not the offered provision, for he desired to follow an independent profession, and the result of many interviews was that he determined to enter Lincoln's Inn as a student of Law, whilst giving some morning hours to his uncle's work, dining with him twice a week, and writing for him after dinner, from 8 to 11 p.m. In one capacity or another he acted as his uncle's secretary until 1832, when the death of the latter, in many of whose ideas he did not participate, released him from his irksome labour, without however fulfilling his just expectations of reward; for, owing to the many fruitless speculations of the great jurist, the sums squandered by his executors on the posthumous publications of his works, and some irregularities in his will, Bentham benefited chiefly by coming into pos-

session of the house in Queen Square Place[1]. Here, after his marriage in 1833 to a daughter of the Rt. Hon. Sir Harford Brydges, Bart., formerly H. M. Envoy at the Court of the Shah of Persia, he resided until 1842. He had, however, been rendered independent through his father's death two years previously.

The amount and variety of mental work achieved by Bentham, during the years of bondage to his uncle, is very remarkable in many ways. Over and above his duties as his uncle's secretary, he had to arrange, often rewrite, and edit, his father's voluminous papers on the administration of the dockyards and other naval matters, and to study law. His legal studies were finally abandoned for logic and jurisprudence, but not till after he had published three notable papers; one on codification, on which subject he entirely disagreed with his uncle, but the paper attracted the attention of Brougham, Hume, and O'Connel. Another paper was on the laws affecting larceny, apropos of Sir Robert Peel's bill for the consolidation of the Criminal Law. Of this Peel thought so highly that he complimented its author, and informed him that it should be submitted to Sir John Richardson, to whom the bill was referred; a copy of it being shown by his uncle to Lord Brougham, the latter wrote a letter of eighteen pages of remarks upon it. The third was a pamphlet on the Law of Real Property. But his most considerable work of this period received scant attention from those most interested in its subject, and passed from its birth directly into an oblivion from which it was rescued only in later years, yet without word or sign from its author. This was his 'Outlines of a new system of Logic, with a critical examination of Dr. Whately's Elements of Logic,' published in 1827. In it the Quantification of the Predicate[2] was

[1] It overlooked St James's Park and the parade ground of Wellington Barracks, and its site is now approximately occupied by the 'Bentham wing' of the Queen Anne's Mansions. It had been in possession of the family for upwards of a century, having been purchased by Bentham's paternal grandfather.

[2] The following history of this episode in Bentham's career is, I think, too interesting and too important to be omitted in his obituary. It is taken from the

first systematically applied in such wise that Professor Stanley Jevons declared it to be 'undoubtedly the most fruitful discovery made in abstract logical science since the time of Aristotle.' Meanwhile Bentham had been called to the Bar by Lincoln's Inn, but his career as a barrister was the briefest. As counsel in a case he broke down in court through nervousness, and thenceforth wisely abandoned the practice of the profession.

In botany Bentham was more at home than in the Law-courts. In 1828 his herbarium arrived from France, and in the same year he was elected a Fellow of the Linnean

Memorial by Professor Gray: 'Before sixty copies of the work had been sold, the publishers became bankrupt, and the whole impression of this work of a young and unknown author was sold for waste paper. One of the extant copies, however, came into the hands of the distinguished philosopher, Sir W. Hamilton, to whom the discovery of the quantification of the predicate was credited, and who, in claiming it, brought an acrimonious charge of plagiarism against Professor De Morgan upon this subject: yet this very book of Mr. Bentham's is one of the ten placed by the title at the head of Sir William Hamilton's article on Logic in the Edinburgh Review for April, 1833, and is once or twice referred to in that article; and a dozen years later, in the course of the controversy with De Morgan, Sir William alluded to the article, as containing the germs of his discovery. We may imagine the avidity with which De Morgan, injuriously attacked, would have seized upon Mr. Bentham's book if he had known of it. It is not so easy to understand how Mr. Bentham, although then absorbed in botanical researches, could have overlooked the controversy in the Athenaeum, or how, if he knew of it, he could have kept silence. It was only at the close of 1850 that Mr. Warlow sent from the coast of Wales a letter to the Athenaeum, in which he refers to Bentham's book as one that had long before anticipated this interesting discovery. Although Hamilton himself never offered an explanation of his now unpleasant position (for the note obliquely referring to the matter in the second edition of his Discussions is not an explanation), Mr. Bain did (in the Athenaeum for Feb. 1, 1851); he immediately endeavoured to discredit the importance of Bentham's work, and again in 1873 (Contemporary Review, Vol. xxi) in reply to Herbert Spencer's reclamation of Bentham's discovery. To this Stanley Jevons made reply in the same volume (pp. 821–824); and later in his Principles of Science (ii. p. 387) this competent and impartial judge, in speaking of the connexion of Bentham's work "with the great discovery of the Quantification of the Predicate" adds, "I must continue to hold that the principle of quantification is explicitly stated by Mr. Bentham; and it must be regarded as a remarkable fact in the history of logic, that Hamilton, while vindicating in 1847 his own claims to originality and priority as against the scheme of De Morgan, should have overlooked the much earlier and more closely related discoveries of Bentham. It must be that Hamilton reviewed Bentham's book without reading it through, or that its ideas did not at the time leave any conscious impression upon the reviewer's mind, yet may have fructified afterwards."'

Society, the meetings of which, the anniversary dinners, and those of its club, he punctually attended. Soon after this Robert Brown proposed his name for election by the Royal Society, but withdrew it before the day fixed for election, to mark the dissatisfaction on the part of the scientific Fellows with the management of the Society, when a Royal Duke was made President. It was not until 1862 that he was again proposed and elected.

In 1829, at the joint solicitation of his friend Mr. Joseph Sabine, the Hon. Secretary, and Dr. Lindley, Assistant Secretary, who were at issue as to the management of the Horticultural Society, he accepted the honorary secretaryship himself, and held it until 1840. On his entering office the Society was in a perilous position from debt and dissensions, from which, with Lindley's active co-operation, he rescued it. It was during his term of office that the celebrated Chiswick Horticultural fêtes were inaugurated, which gave a new life to the science. At the first of these, held on April 3, 1832, seventeen hundred persons were present. It was during the same period that so many of our most popular garden-plants were introduced, especially from California, through collectors sent out by the Society (Douglas, Hartweg, and others). These plants were named, and many novelties amongst them described, by Bentham in the Society's publications; to which he also contributed a translation of Targioni-Tozetti's 'Historical Notes on Cultivated Plants,' in which he added much valuable matter to the author's work. At about this time Dr. Wallich returned from India with his enormous collection of Himalayan, Burmese, and Indian plants, destined to be named and distributed to the principal herbaria in Europe by the Honourable East India Company. In furtherance of this great work Bentham offered his aid to Dr. Wallich, with whom he co-operated zealously for several successive years. Over and above his gratuitous labours as an assistant, he undertook the naming and distributing of the orders Euphorbiaceae and Gramineae, lithographing the tickets of the latter with his own hand. This marks an epoch in

his career, for it was his introduction to a tropical flora (which he was never privileged to see), and afforded the chief materials for his first work on exotic plants, the 'Scrophularineae Indicae,' published in 1835. This was followed, in 1836, by the completion of his great work, 'Labiatarum genera et species, or a description of the genera and species of the order Labiatae, with their general history, characters, affinities, and geographical distribution,' which gave him a position in the very foremost ranks of taxonomic botanists[1]. It was followed, in 1837, by his enumeration of plants collected in the Swan River district by Baron Hugel (Vienna, 1837), which is remarkable as showing the ease and rapidity with which he mastered a Flora totally different from those he had previously studied.

From the summer of 1836 till the early part of 1837 he resided with his wife in Germany, visiting the principal Botanic Gardens and Herbaria, especially engaged in studying the order Leguminosae; his account of these and of the botanists whom he met was communicated in letters to Sir W. Hooker, and published (anonymously) in the Companion to the Botanical Magazine (Vol. ii) and Journal of Botany (Vol. i). They are very interesting as contributions to the History of Botany in the first third of the century. During the winter at Vienna he published his masterly 'Commentationes de Leguminosarum generibus' in the 'Annalen des Wiener Museums' (Vol. ii).

In 1846–7 he undertook, accompanied by his wife, an extended tour in Europe. Commencing with Hamburg, they visited Copenhagen, Stockholm, St. Petersburg, Moscow, Odessa, Constantinople, Trieste, Bologna, Florence, Leghorn, Naples, Rome, Palermo, and Geneva. What he saw in these towns and their environs of botanical, horticultural, and other

[1] To give some idea of the thoroughness of Bentham's methods, it is well to state, that in prosecution of this work he visited the following herbaria:—in 1830 Hamburg and Berlin; in 1831 Paris, Geneva, Avignon, and Montpellier; in 1832 Hamburg again, Copenhagen, Leipzig, Dresden, Prague, Vienna, and Munich; in 1833 Paris and Montpellier again; and in 1834 Bonn, Frankfort, Geneva again, Pavia and Turin (Labiat. Gen. and S., Pref. p. v).

interest was communicated in thirty-one anonymous letters to the Gardener's Chronicle (Vols. 1846–7). Like those mentioned above from Germany, they are of great value as contributions to the history of botany and horticulture during the period in which they were written.

With the view of providing better accommodation for his library and herbarium, and devoting himself exclusively to science, Bentham removed in 1842 to Pontrilas, an Elizabethan manor house belonging to his brother-in-law, Colonel Scudamore. Here his chief occupation was providing material for the continuation of Auguste De Candolle's 'Prodromus systematis naturalis Regni Vegetabilis,' which had been undertaken by his (Bentham's) intimate friend, Alphonse De Candolle. In this work he contributed the Ericeae, Polemoniaceae, Scrophularineae, Eriogoneae, and a greatly enlarged revision of the Labiatae, amounting in all to over 4,730 species. During the same interval he published the Botany of the Voyage of the Sulphur in the Malayan seas and Pacific ocean, a quarto work with 60 plates.

Whilst resident at Pontrilas he also did his duty as Justice of the Peace for the county of Hereford with punctuality and efficiency.

In 1854, finding that the cost of keeping up his library and herbarium threatened to exceed his income, he determined to offer these to the Government, with the stipulation that they should form part of the establishment of Kew[1], he himself abandoning botany, and removing from Pontrilas to London. This munificent offer was of course gladly accepted by the Government, and the materials were placed in what is now called the Herbarium building of Kew, to be subsequently amalgamated with the richer collections of books and plants

[1] Where there were at that time no other library or herbarium than the private ones of Sir W. Hooker, which he had been permitted to deposit in a house previously in the occupation of one of the Royal Family. The said house had, at the advice of Sir Joseph Banks, been purchased by George III for the very purpose which it now serves, and one room was actually shelved for the books. On the death of the king and his scientific counsellor, in the same year, the house was otherwise appropriated.

of Sir W. Hooker. On the other hand, the idea of Bentham's giving up botany was a shock to his scientific friends at home and abroad, and especially to the oldest and most intimate of them, Sir W. Hooker, who begged him to reconsider his intention, combated his own modest estimate of himself as a mere amateur systematist, and pointed out to him that a residence in London offered the means of study at Kew, where a room, containing his own herbarium, should be devoted to his use, and where he would be in proximity to the garden, museum, and collections already at Kew. Fortunately Sir W. Hooker's counsels, backed by those of other friends, especially Dr. Lindley, prevailed. In 1855 he took up his residence in London, for the first few years in Victoria Street, Westminster, and for the remainder of his life in 25 Wilton Place, between Hyde Park and Belgrave Square. From London he went to Kew daily (a few weeks of autumn holidays excepted) for five days a week, with perfect regularity, arriving at 10 a.m. and leaving at 4 p.m., devoting the evenings to writing out the notes of his day's work, and never breaking the long fast between breakfast at 8 or 8.30 and dinner at 7.30 or 8. 'With such methodical habits, with freedom from professional or administrative functions, which consume the time of most botanists, with steady devotion to his chosen work, and with nearly all authentic material and needful appliances at hand or within reach, it is not surprising that he should have undertaken and have so well accomplished such a vast amount of work, and he has the crowning merit and happy fortune of having completed all that he undertook' (A. Gray, Memorial).

No sooner was Bentham settled within reach of Kew than he was induced by Sir W. Hooker to inaugurate a series of Colonial Floras, which had been planned by the former, and of which the first is that of Hong-Kong[1]. It was followed by the 'Flora Australiensis,' in seven volumes, which is the first flora of any large continental area that has ever been

[1] The 'Flora Hong-Kongensis,' published in 1861, one vol. 8vo, pp. 455, contains 1,103 species.

finished. It was commenced in 1861, and was concluded in 1870; it comprises about 7,000 species. He was aided in it by valuable notes and preliminary studies supplied by Baron Mueller, but every description, generic and specific, was strictly his own. As has been well said, 'it is a work which would alone found a reputation.'

But Bentham's *magnum opus* is unquestionably the 'Genera Plantarum[1],' issued under the joint authorship of himself and the contributor of this memoir to the Annals; but which, whether for the overwhelming share of the work which Bentham undertook, or for the aid he gave his partner in certain Orders elaborated by the latter, may justly be regarded as on the whole the product of one botanist. In the planning and execution of the work only two points were contested between us, whether his or my name should take precedence on the title-page, and whether in the headings of the pages the author's name should be given with that of the Order described. On the first point my opinion prevailed, his on the second.

The only other separate work published by Bentham during this period was an Illustrated Handbook of the British Flora, for the use of beginners and amateurs, including a series of wood-engravings, with dissections, by W. H. Fitch, F.L.S. The first edition of the Handbook appeared in 1858, and has been succeeded by five others. This work, on its appearance, was criticized on the false assumption that its author had no knowledge of plants in the field, supported by the fact that he took a much wider view of the variations under which species present themselves in nature, than do authors who have that knowledge. It was unknown to, or forgotten by,

[1] Genera Plantarum ad Exemplaria, imprimis in Herbariis Kewensibus servata, definita, auctoribus G. Bentham et J. D. Hooker, 1862-1883. The last article which Bentham wrote was a communication to the Linnean Society (Proc. xx, 1883, p. 304): On the joint and separate work of the authors of Bentham and Hooker's 'Genera Plantarum'; where a full account is given of the part each author took. It may be mentioned that with the view of reducing the price of the work to the public as far as possible, the expense of production was defrayed by the authors, Mr. Bentham guaranteeing that his fellow author should not lose.

his critics, that he had for half-a-century observed, collected, and preserved most of the species of the British Flora over a great part of Continental Europe, as well as in the British Isles, that his views were founded on wide experience, and that the results of them in terms of genera and species were drawn up from examination, in almost every case, of living examples. The Handbook was a great favourite of Mr. Darwin, whose admiration of the masterly way in which the author dealt with the main features of the British Flora, drew from him the exclamation, 'Good Heavens! to think of British botanists turning up their noses and saying that he knows nothing of British plants[1].'

Bentham's labours at Kew on the above three works were interrupted by several serious demands on his time and energies, his response to which places in a striking light his disinterested devotion to the progress of science. Of these the most important was his acceptance of the Presidency of the Linnean Society. This he accepted in 1863, and threw himself into the duties of the office, which he discharged for eleven years, with energy, wisdom, and singleness of purpose; and, it should be recorded, with no small expenditure of his means. It made no difference in respect of the time devoted to his work at Kew; for the one day of the week which he had reserved for his own affairs was thereafter devoted wholly, or in part, together with much of his evening hours, to the Society's affairs. During the years in which he held office he took the chair at the evening and council meetings, with all but unbroken punctuality. On the transference of the Society's library, collections, and portraits, to the apartments in Burlington House provided by the Government, he personally superintended the arrangements, classifying the books, and literally with his own hands placing them on the book-shelves; and he himself indexed

[1] Life and Letters, Vol. ii, p. 363. It must not be supposed that Bentham disparaged the labours of those who aimed at what he considered the multiplication of species. No naturalist was more appreciative of accurate work in this department of botany, and of its value.

the first ten volumes of the Transactions. He constituted himself the editor of most of the botanical papers published in the Transactions and Journal, in some cases earning the gratitude of the authors by rearranging their matter (with their approval), and himself rewriting their papers. His annual presidential addresses were remarkable for their wide range of knowledge, and those who knew him only as a systematist and descriptive botanist recognized with surprise the power of analysis and sound judgment which he displayed in these addresses, wherein he discussed evolution in all its bearings, the writings of Haeckel, geographical distribution, the prospects of fossil botany, deep sea life, abiogenesis, methods of biological study, the histories and labours of the principal Natural History Societies, and periodicals of every civilized country on the globe.

In respect of evolution, perhaps the most important of his addresses is that of 1863, dealing with discussions on the Origin of Species. Alluding to his own subsequent tardy adoption of the theory of the Survival of the Fittest, he says: 'I scarcely think that due allowance is made for those who, like myself, through a long course of study of the phenomena of organic life, had been led more or less to believe in the immutability of species within certain limits, and have now felt their theories rudely shaken by the new light opened on the field by Mr. Darwin, but who cannot surrender at discretion so long as many important outworks remained contestable.' In correspondence with Mr. Darwin on some of these outworks, the latter in a letter dated June 19, 1863, alluding to the effect of the address as a whole, wrote[1]: 'I verily believe that your address, written as it is, will do more to shake the unshaken and bring in those leaning to our side than anything written in favour of transmutation.' It is interesting to find in later addresses, a frank acceptance of evolution, in such passages as those in which he recognizes 'the coexistence of indefinite permanency, and of gradual or rapid change in different races in the same

[1] Life and Letters, Vol. iii, p. 26.

area, and under the same physical conditions'; and, 'we must now test our species as well as genera or other groups, by such evidence as we can collect of affinity derived from consanguinity.' In short, as with Lyell in the later editions of his famous Principles of Geology, when dealing with the history of life on the globe, Bentham had to underpin his edifices, and replace their old foundations with new. Happily in the cases of both philosophers, this was effected without injury to the superstructures.

This brief notice of Bentham's final adhesion to Darwin's views may be supplemented by the following interesting extract from a letter he wrote to Francis Darwin[1], May 2, 1882 (two years before his death). It says:—'I have always been throughout one of his (Darwin's) most sincere admirers, and fully adopted his theories and conclusions, notwithstanding the severe pain and disappointment they at first occasioned me. On the day that his celebrated paper was read at the Linnean Society, July 1, 1858, a long paper of mine had been chosen for reading, in which, in commenting on the British Flora, I had collected a number of observations and facts illustrating what I then believed to be a fixity of species, however difficult it might be to assign their limits, and showing a tendency of abnormal forms produced by cultivation or otherwise to withdraw within their original limits when left to themselves. Most fortunately my paper had to give way to Mr. Darwin's, and when once that was read, I felt bound to defer mine for reconsideration; I began to entertain doubts on the subject; and on the appearance of the Origin of Species I was forced, however reluctantly, to give up my long cherished convictions, the results of much labour and study, and I cancelled all that part of my paper which urged original fixity.' This paper of Bentham's was never published.

Of the many laborious tasks undertaken and gratuitously performed by Bentham, chiefly at Kew, the following deserve especial notice. First and greatest was the equipment of

[1] Life and Letters, Vol. ii, p. 293.

the University of Cambridge with an authentically named consulting herbarium. This consisted for the most part of that of his friend, Dr. C. Leman, F.L.S., a zealous collector, especially by purchase, which he was disposed to leave by will to Bentham. The latter, on the other hand, urged its being left to Cambridge, of which Dr. Leman was a graduate in medicine. It was finally arranged between them, that on his friend's death the collections should be sent to Bentham, who should select from them any specimens which he might want for his own herbarium, whilst the remainder (the much larger portion), augmented by duplicates from Bentham's herbarium, should go to Cambridge. Aided by a small grant from the University for the purchase of paper, for the expenses of mounting and poisoning the specimens, and for other contingencies, Bentham classified, named, had fastened down and enclosed in genus-covers, a consulting herbarium of 30,000 species. This great labour occupied more or less of ten years of his life. Other gratuitous tasks were the ticketing, and dividing into sets for sale, of the collections of Robert and Richard Schomburgk in Guiana and Brazil, and of Hartweg[1] in British Columbia, California, and Mexico. A still greater service to science was his undertaking the distribution and sale of the magnificent collections of the distinguished traveller Richard Spruce in the Amazon region and Peru. These, amounting in all to 6,500 numbers, were sent to him as collected, to be arranged, named, and divided into twenty to thirty sets, for which he obtained subscribers in the principal public and private museums in Europe and America. He further collected the money due by the subscribers, transmitting it to Spruce, who depended on it for the prosecution of his thirteen years of exploration, thus saving to all parties the expenses of agency and commission.

Bentham's last work was the 'Genera Plantarum,' of

[1] Of Hartweg's plants he published a catalogue, with descriptions of new genera and species under the title of 'Plantae Hartwegianae.' It enumerates about 1,400 species.

which the first part appeared in July 1862, the concluding in April 1883. The closing years of his life are feelingly described by Mr. Thiselton-Dyer in his Eulogium, in the following words:—'In the latter years of his life Bentham was not less imbued with affection for his task, though the sense of the precariousness of life chiefly affected him with anxiety as to its completion. The flame of his intellectual powers never burnt more brightly, too brightly perhaps for a frame which slowly but perceptibly enfeebled. During the last years of what was a supreme effort, it was impossible not to feel a degree of awe for the intense devotion with which he pursued, without intermission, his self-imposed labour. Towards the last it appeared to one that by mere effort of will he actually sustained his bodily vitality. When the last revise of the last sheet[1] was returned to the printer, the stimulus was withdrawn. Nature, so long indulgent, would no longer be withstood. He came once or twice again to Kew, but found no task that he could settle to. At home he commenced a brief autobiography. The pen[2] with which he had written his two greatest works broke in his hand in the middle of a page. He accepted the omen, laid aside the unfinished manuscript, and patiently awaited the not distant end.'

I cannot better conclude this attempt to convey an adequate idea of the value and amount of Bentham's labours, than by citing a passage from his intimate friend's, Dr. Asa Gray's, Memorial, premising that the latter botanist most nearly approached him of all his scientific contemporaries in the qualities he alludes to and the range of his work. He writes:—'It will have been seen that Mr. Bentham confined himself to the Phanerogams, to morphological,

[1] This was the general index, which, as those of each successive part, he made himself, so scrupulous was he in his efforts to avoid error, even in so mechanical an operation.

[2] This was one of Mordan's gold pens, which have I think iridium nibs. It 'wrote' not only the seven volumes of the 'Flora Australiensis,' and the three of the 'Genera Plantarum,' but a vast number of botanical papers and letters. The pen-holder is preserved at the Herbarium, Kew.

taxonomical, and descriptive work, not paying attention to the Cryptogams below the Ferns, nor to vegetable anatomy, physiology or palaeontology. He was what may be called a botanist of the old school. Up to middle age, and beyond, he used rather to regard himself as an amateur, pursuing botany as an intellectual exercise. "There are diversities of gifts," perhaps no professional naturalist made more of his, certainly no one ever laboured more diligently, nor indeed more successfully over so wide a field, within these chosen lines. For extent and variety of good work accomplished, for an intuitive sense of method, for lucidity and accuracy, and for insight, George Bentham may fairly be compared with Linnaeus, De Candolle, and Robert Brown.' This is a just tribute to his memory, to which I would add my own, that method, grasp of subject, and thoroughness, were his watchwords.

It remains to allude to his personal characteristics. He was tall, of spare habit, with a slight stoop in his gait; his features were strongly marked, his complexion rather dark, his hair black and eyebrows bushy. The likeness[1] accompanying this memoir is an excellent one. In dieting himself, he was extraordinarily abstemious, taking but two meals a day, and those most sparing. Though shy and reserved in manner, he was a most amiable, warm-hearted man, the kindest of help-mates, and the most disinterested of friends. As a companion or guest he was charming, high bred, and courteous, communicative of stores of anecdotes and reminiscences of the events he had witnessed, the interesting people he had known, and the places he had seen all over Europe. To which must be added his musical gift, which was at the service of whoever asked for it. To recognitions and honours he was indifferent. He gratefully received the Royal Medal from the Royal Society, awarded in 1859, and the Clarke Medal of the Royal Society

[1] It is a reproduction of the portrait, painted in 1870 by Lowes Dickinson, in the possession of the Linnean Society of London, by whose kind permission it has been here reproduced.

of New South Wales in 1878; but it was with great difficulty that he was prevailed upon to receive the Companionship of St. Michael and St. George, conferred on him by Her Majesty. He was a correspondent of the Institute of France, and member of the Academies of Science of Berlin, St. Petersburg, and America, and of many other scientific Societies that cultivate Natural History.

Bentham died of old age at his house in Wilton Place, December 10, 1884, shortly after his eighty-fourth birthday, retaining his faculties to the last. His wife predeceased him by four years; he never had any family. The bulk of his modest fortune went to his only relative, a great-niece residing in France, after liberal bequests to the Linnean Society and the scientific relief fund of the Royal Society, and of a considerable sum under trust to be expended in the interest of the herbarium at Kew, especially in continuing the publication of Hooker's Icones Plantarum, a work in which he took a great interest, having indeed provided the plates and letter-press of several volumes at his own expense.

J. D. HOOKER.

The Development of the Flower and Embryo in Lilaea subulata, H.B.K.

BY

D. H. CAMPBELL, PH.D.,

Professor of Botany in the Stanford University, California, U.S.A.

With Plates I-III.

THE genus *Lilaea* is a peculiar monotypic one, the single species, *L. subulata*, being widely distributed through the western part of the American continent. According to Hieronymus[1], who has made the most careful study of the plant, its range is from Oregon, throughout the coast region of California and Mexico, into South America. In the latter it has been collected in Colombia, Chile, Argentina, and Uruguay, and has been found at various elevations, from sea-level to a height of 3,000 metres.

There is much diversity of opinion as to the systematic position of the plant. In Engler and Prantl's Natürliche Pflanzenfamilien[2], it is classed with the Juncaginaceae; but Schumann[3] is inclined to consider it as representing a special family, Lilaeaceae, proposed originally by Hieronymus, and

[1] Engler and Prantl, Die Natürlichen Pflanzenfamilien, II, 1, p. 225.
[2] Loc. cit.
[3] Morphologische Studien, Leipzig, 1892, Heft I, p. 187.

[Annals of Botany, Vol. XII. No. XLV. March, 1898.]

this probably is more in accordance with the peculiar characters of the plant.

Our knowledge of the morphology of the plant is mainly derived from the elaborate monograph of Hieronymus[1], which proposed to give a very full account of the morphology, but was unfortunately left incomplete. Schumann[2] has given some details as to the relation of the flower to the axis of the plant, but these simply confirm the earlier observations of Hieronymus. Beyond the work of these observers, so far as the writer knows, the plant has been described only in a superficial way.

The writer has been engaged for some time upon a study of the flower and embryo in a number of the simpler Monocotyledons, and among the forms which have engaged his attention is *Lilaea*, which is common in the region about San Francisco Bay. The results of these studies are given in the following pages.

The material upon which these were made was for the most part collected in the neighbourhood of Stanford University, where the plant is a common one. In this neighbourhood the plant grows either in shallow water, or completely exposed upon the mud. More rarely the plant is completely submerged except the flowers. It is an annual, germinating with the advent of the winter-rains, and flowering within a few weeks of germination. Flowers continue to form as long as the plant grows, but the plant is finally killed by the drying up of the mud in which it is rooted. The ripened fruits remain in the dried mud during the summer and autumn, and germinate as soon as the rains have soaked the ground.

Most of the plants collected by the writer grew in the tenacious black clay ('adobe') characteristic of much of the land in the immediate vicinity of the University. The favourite localities for the plant were depressions in the fields

[1] Monografía de Lilaea: Actas de la Academia Nacional de Ciencias en Cordoba. Buenos Aires, 1892.

[2] Loc. cit.

where the water collected and formed shallow pools. These seemed to offer the most favourable conditions for the germination of the seeds. Of course the time of germination varies with the time of the rains, but in January, 1897, the plants were found in various stages of germination; and in 1895, an unusually rainy winter, the plants were well in flower in February.

The general aspect of the plant is curiously like *Isoetes*, the subulate leaves forming dense tufts from the short, bulb-like stem. The leaves are strictly two-ranked, and numerous white unbranched roots fasten the plant into the soft mud. The arrangement of the leaves and roots, as well as that of the flowers, has been given in detail by Hieronymus[1], and will be discussed only briefly here. The flowers are exceedingly simple in structure, but nevertheless show much variation, at least in the pistillate flowers which exhibit heterostylism in a very remarkable degree.

After the young plant has produced several—usually from five to seven—leaves, the axis becomes transformed into a shaft bearing a spike of flowers; while in the axil of the last-formed leaf is formed a shoot which, after producing a single leaf, is transformed also into an inflorescence like that derived from the original stem-apex, and in the axil of the leaf is formed another shoot which behaves in the same way. Thus the branching of the plant is sympodial. In addition to the flowers borne upon the spike, there are usually found two close to the base of the shaft but not outgrowths of it. These basal flowers are always pistillate, and are distinguished by their extremely long styles, which may reach a length of several centimetres (14 c. in some cases, according to Hieronymus).

The lowest flowers of the spike, where these are pistillate, are destitute of any subtending bract or leaf, but the other flowers are borne in the axil of a small bract. Of the pistillate flowers borne upon the spike, the lower ones are

[1] Monografia de Lilaea.

intermediate in character between the very long-styled basal flowers and the very short-styled flowers near the apex, in which the stigma is nearly or quite sessile.

In the axils of all the foliage-leaves are borne a number of the delicate scales (*squamulae intravaginales*) so characteristic of most of the simple aquatic Monocotyledons.

The histology of the vegetative organs of the plant has been worked out very carefully by Hieronymus: but unfortunately only the roots and leaves are fully described, his memoir ending abruptly before the description of the stem was completed. Numerous beautiful and accurate figures of the histology are however given, which make it possible to follow out most of the structural details.

In common with other aquatics, the leaves contain large lacunae, which here are irregularly arranged, and separated from one another by single layers of cells. There are numerous vascular bundles of the ordinary collateral type. The number of these bundles varies much with the size of the leaves. Hieronymus[1] states that in the most vigorous leaves he studied there were sometimes twenty-one. Of these one occupies a nearly median position, near the outer side of the leaf, and he considers this to represent a midrib.

The epidermis of the leaves shows the usual elongated cells of similar leaves, and stomata are found, characterized by the presence of accessory cells not unlike those of many Grasses.

The author's own observations were based mainly upon series of microtome-sections. The material was fixed with chromic acid, and, after thorough washing, stained *in toto* with Czokor's alum-cochineal, and afterwards stained on the slide with alcoholic Bismark-brown. For the study of the embryo-sac, safranin and haematoxylin were also used to some extent as nuclear stains.

[1] Monografía de Lilaea, p. 39.

General Morphology of the Young Plant.

In Fig. 2 is shown a median longitudinal section of a young plant with the apex prolonged into the first floral spike (×), and the two basal pistillate flowers (♀) already formed. The stem itself is very short, and made up of nearly uniform parenchymatous tissue, which is traversed by the vascular bundles running into the leaves and the young inflorescence; short bundles are also given off to the basal flowers. The arrangement of the parts in a more advanced stage can be seen in Fig. 1, where several secondary inflorescences have formed.

The number of roots is very large. According to Hieronymus, there is usually a secondary root on each side of the cotyledon, and the number formed from the later nodes varies with the size of the plant. No special study was made of the origin of these secondary roots, beyond noting that they form deep down within the tissue of the stem near the base of the leaves and close to a vascular bundle. They are consequently well developed before they finally break through the overlying tissues. The structure of the roots and the arrangement of the primary tissues are only briefly treated by Hieronymus[1], but he gives very accurate figures which make it perfectly clear that the arrangement of the tissues is that of the typical Monocotyledons, and these points have been confirmed by the writer. The very distinct plerome-cylinder, showing about five rows of cells in longitudinal section, is separated from the root-cap by a group of cells which are the common initials for epidermis and cortex. There is a separate group of initials for the root-cap. Hieronymus' figures would indicate a single initial cell for the plerome, but he has not stated this in the text; my own preparations make this seem not improbable, but it was not possible to decide the matter positively.

The number of squamulae is variable, and they do not

[1] Monografia de Lilaea, Pl. IV.

differ in any marked degree from similar structures in other Monocotyledons. The cells have delicate walls, and densely granular protoplasm with a distinct nucleus. The whole aspect of the cells is that of secretory ones, but this point was not further investigated.

Development of the Inflorescence.

The primary inflorescence, as we have seen, is the direct prolongation of the original stem-apex of the young plant, and the later ones have a similar relation to the secondary shoots, which first produce a single leaf and then elongate at once to form the inflorescence. The apex gives rise to a stamen, while the other flowers—except the basal pistillate ones—are formed as lateral appendages. The lowermost flowers are usually female, with a moderately long style, and without any subtending bract; but the other flowers have below them a small bract, which probably is the equivalent of the leaf at the base of the main shoots.

The arrangement of the flowers upon the inflorescence shows a good deal of variation. The commonest arrangement upon well-developed spikes is that in which the lowest flowers are pistillate, the central ones hermaphrodite—or probably a secondary inflorescence made up of two flowers, male and female,—and those at the apex are staminate only. The writer has, however, seen cases where all the flowers were staminate, each stamen being subtended by a bract; and Hieronymus[1] figures a specimen where only female flowers were developed. In the latter case, to judge from his figures, all the flowers were destitute of bracts. Where a single flower only is produced in the axil of the bract, the primordium or young shoot is transformed directly into the carpel or stamen, as the case may be; but when the carpel and stamen are formed together, there is a division of the primordium into two equal parts, and this appears

[1] Monografía de Lilaea, Pl. I, Fig. 6.

to be a true dichotomy like that which occurs in *Naias* previous to the formation of the flower.

In Fig. 3 is shown a median section through the floral complex from the middle region of a very young inflorescence. The arrangement of the organs suggests very strongly that found in *Naias* at a similar stage of development [1]. This consists of two nearly equal superimposed protuberances, respectively ♂ and ♀, which apparently are formed by a true dichotomy of a common primordium. The latter has below it a very slightly projecting ridge, *l*, the rudiment of the subtending bract found in the older flowers. Of the two parts into which the primordium is divided, the upper is bluntly conical in form, the lower more pointed when seen in section, but really considerably flattened in the plane of division of the primordium. The upper prominence is the young carpel, the lower the stamen. Each of these structures, from analogy with the very similar ones in *Naias* and *Zannichellia*, may be considered as representing shoots of equal morphological value, resulting from the dichotomy of a common primordium. If this view is accepted, both the stamen and carpel must be considered as truly axial structures.

The young primordium is composed of nearly similar cells, which are arranged in the manner characteristic of the stem-apex of the Angiosperms. There is a definite dermatogen below which is a pretty clearly defined layer of periblem which separates the dermatogen from the central plerome-mass. This arrangement is especially clear in the staminal rudiment.

The Male Flower.

The development of the single stamen which constitutes the male flower, whether formed alone in the axil of the bract, or secondarily from the dichotomy of the axillary primordium, follows the same line of development.

[1] Magnus, Beitrage zur Kenntniss der Gattung Naias; Berlin, 1870. Campbell, A Morphological Study of Naias and Zannichellia, Proc. California Academy of Sciences, 1897

The very young stamen, cut parallel to its broad face (Fig. 5), is broadly conical, slightly constricted at the base, thus indicating the differentiation of anther and filament. In this stage it is composed of nearly isodiametric parenchyma, but shows the definite divisions of the tissues into dermatogen, periblem, and plerome, referred to in connexion with the original primordium. The plerome in this view has a broadly conical form, with two lateral outgrowths marking the position of the future loculi. A cross-section of a similar stage shows that there are really four of these.

A cross-section of a somewhat older stage is shown in Fig. 7. While there is in general the same arrangement of the tissues seen in the younger stamen, the loculi are more clearly defined. The two upon the outer side are somewhat broader than the others, and between them there is a small sterile lobe. Each loculus shows much the same arrangement of the tissues as the whole of the young staminal rudiment, and the origin of the sporogenous tissue is plainly traceable in all cases to the plerome. The latter is usually well defined, and forms a conical mass, usually three or four cells wide at the base, and narrowing above. The periblem, which at first is but one cell thick, later, by the formation of periclinal walls, becomes thicker, and finally the limits between it and the plerome are no longer distinguishable. In cross-section at this stage, there may be seen two vascular bundles near the base of the inner loculi. The exact origin of the sporogenous cells is difficult to trace, but there seems no question that they originate from the outer cells of the plerome, and that the archesporium is not of hypodermal origin as is usually the case. In this respect *Lilaea* agrees with *Naias flexilis*, and probably also with *Zannichellia*, although in the latter the question is not quite clear[1].

At first the young sporogenous cells are not readily distinguishable from those surrounding them, and it is quite impossible to trace them back certainly to the division of

[1] Campbell, l. c., pp. 13 and 41.

a single primary archesporial cell; indeed it is quite improbable that they all originate from a common mother-cell. As they become older, however, they become noticeably larger than their neighbours, and show the usual dense contents and large nuclei (Figs. 9, 10). However, even in these stages the transition from genuine sporogenous tissue to the tapetal cells is somewhat gradual. The latter are derived mainly from the periblem, but functionally, at least, some of the plerome-cells must be regarded as tapetal.

As the stamen grows, the four masses of sporogenous tissue become very clearly defined. Each group of sporogenous cells is surrounded by about three, or in places four or five, layers of cells which separate it from the epidermis. The cells, which at first are much alike, later show considerable differentiation.

Fig. 10 shows a longitudinal section of a loculus shortly before the separation of the sporogenous cells. The epidermal cells are now much elongated, but are very little deeper than they were in the very young stamen. Under the epidermis is a layer of somewhat swollen cells which, with the epidermis, persists as the wall of the mature loculus. Within this second layer is a third one, composed of very much compressed cells (*c*), which with a fourth layer (*d*) make up the tapetum. The layer *d* has larger cells which resemble the sporogenous cells in the character of their contents.

The sporogenous cells (*sp.*) have the usual characters of such cells. They are thin-walled, isodiametric, with abundant granular cytoplasm and large nuclei. The nucleoli are conspicuous and the chromatin abundant. The nucleus, in material fixed with chromic acid, usually shows a conspicuous areola about the nucleolus. On the inner side of the loculus especially, the tapetal cells encroach upon the sporogenous area, and there are cells which are intermediate in character between the perfect sporogenous cells and those of the tapetum. These are probably to be considered as potentially sporogenous cells which do not, however, develop into spores, but become broken down and serve, like the true tapetal cells,

to nourish the developing spores. A similar disintegration of part of the sporogenous tissue has been observed by the writer in *Zannichellia*, and is very much like what takes place in *Equisetum*.

The sporogenous cells after separation are imbedded in a nucleated mass of protoplasm derived from the tapetal cells and the imperfect sporogenous ones (Fig. 11). As in most Monocotyledons the spores are of the bilateral type, i.e. there are two complete successive cell-divisions of the pollen-mother-cell. The spores do not long remain united in tetrads, but separate completely and assume an almost perfectly globular form. The young spore contains but a single nucleus, but there is later a division into two cells of very unequal size. The ripe spore (Figs. 12–14) shows a double wall, the outer one marked with fine reticulations, the inner one being the delicate endospore. The spore before maturity contains very little granular contents, but these increase rapidly as the spore ripens. The exact nature of the reserve substances in the ripe spore was not investigated. The structure of the anther-wall (Fig. 13) is of the usual type.

The nuclei of the two cells in the germinating spore are very different in appearance. That of the large vegetative cell (Fig. 12, *v*) is large, with but little chromatin and a large nucleolus. The nucleus of the small generative or antheridial cell (*g*), on the other hand, is small, staining strongly and having an inconspicuous nucleolus. No indication of a further division of the antheridial cell, such as occurs in many Monocotyledons, was seen, although it is almost certain that this does occur in the pollen-tube after germination.

The Female Flower.

The homologies of the two pistillate flowers which usually occur near the base of the shaft of the inflorescence are not entirely clear, but they probably represent shoots of the same nature as the innovations which occur in the larger plants, in addition to the shoots formed in the axils of the leaves.

These basal flowers arise on either side of the floral axis (Fig. 2, ♀), and are very early recognizable. In these the young ovule is already evident as a slender prominence whose axial nature is unmistakable, and it represents, with little question, the end of the metamorphosed shoot which constitutes the flower. Sometimes, and perhaps always, the flower has at its base a small bract, in which respect it differs from the pistillate flowers of the spike, which are destitute of a similar subtending bract. The central ovular rudiment (Fig. 15, *o*) is enclosed by a cup-shaped envelope arising from the growth of the surrounding tissue, and this body, the carpel, which is formed precisely as in *Naias*, is probably to be considered as a foliar member. It is of equal height on all sides, and shows no evident dorsi-ventral structure.

The pistillate flowers upon the spike are of two kinds, those which stand alone at the base of the spike, and those which are associated with the male flowers. The former are longer-styled, and are in this respect intermediate between the extremely long-styled ones and the upper short-styled ones. In the lower flowers of the spike, the whole primordium is transformed into the flower; in the upper ones, as we have seen, there is first a dichotomy of the primordium, only one member of which forms the pistillate flower. In both cases, however, the flower is to be looked upon as a transformed shoot, whose apex develops into the ovule, while the carpel represents a foliar appendage of the floral axis.

In a section of the young pistillate flower from the spike (Fig. 17), the ovule is not nearly so conspicuous as in the long-styled flowers. Here the young carpel is developed much more strongly upon the inner side, while upon the outer side it is not clearly distinguishable from the ovular rudiment, which is much less noticeable at this stage than it is in the long-styled flowers. The ovule is not so slender as that of the basal flowers, and more rounded at the end; but by comparing it with a somewhat older one (Fig. 19), it is evident that here too the ovule is the transformed apex of the shoot. The young short-styled flower, at this stage, is

very much like the corresponding stage in *Zannichellia*; indeed the whole development of the flower is very much like that of the latter.

In the long-styled flower, the slender ovular rudiment soon shows a broader and somewhat pointed form (Fig. 16). The pointed appearance is due to a stronger growth at this place, by which the original apex is forced over to one side, this being the first indication of the anatropous form of the older ovule. In the short-styled flower, the young ovule (Figs. 17, 19, *o*) is much blunter from the first, but here also the original apex is soon bent over by the excessive growth upon the outer side of the young ovule. In both forms of flowers, the growth of the carpel is rapid; it soon grows up beyond the top of the ovule, and the margins, which at first are quite free, so that the carpel forms an open cup, approach and finally meet, the ovary-cavity thus becoming completely closed. The carpel is closely appressed to the ovule, and its upper part is extended into the tubular style, the final development of the latter varying much as we have already indicated. The young style is traversed by a narrow open canal, but this later becomes entirely closed by the cohesion of the cells lining it. In the long-styled flower, the style and stigma are perfectly symmetrical; but in the short-styled one, the stronger development of the carpel upon the inner side of the young flower persists, so that the stigma is much more strongly developed upon this side (Fig. 25).

As the ovule develops, the much stronger growth on one side forces the original apex over until it assumes nearly a horizontal position (Figs. 18, 20), and finally it becomes perfectly anatropous (Fig. 25). The first integument becomes evident at a very early period, and forms a shallow cup-shaped structure, more strongly developed on the upper side of the ovule (Figs. 18, 20, *in.*). Not long after this, the second (outer) integument is differentiated, but this is fully developed only upon the outer free side of the ovule (Fig. 21, in^2); while upon the inner side, which is in contact with the funiculus, it is imperfect. The inner integument is

composed, for the most part, of two layers of cells and soon reaches to the top of the nucellus, and later closes over it to form the micropyle. The outer integument remains less developed than the inner one, and barely reaches to its level.

The Style and Stigma.

While there are some minor differences in the development of the style in the long- and short-styled flowers, the structure of the fully developed parts is essentially the same. At an early period in the growth of the flower, the free superficial cells of the upper margins of the carpel become enlarged into papillae (Fig. 25, *st.*), which later reach a great size, and are distinguished also by very dense granular cytoplasm and large nuclei. When the pistil is mature, they form a dense tuft of conspicuous stigmatic hairs.

The narrow canal, which is present in the very young style, soon becomes completely obliterated by the coalescence of the cells forming its walls. These cells, which are the continuation of the epidermal cells which form the stigmatic papillae, much resemble the latter in the character of their contents, although the nuclei are smaller. There are, however, transitional forms in the upper part of the style. Occasional indications of more than one nucleus in these cells were seen, but the matter was not further investigated. These cells together form a very distinct strand of conducting-tissue, which in cross-section is oval, and very clearly defined.

The arrangement of the tissues in the style may be readily seen by comparing cross and longitudinal sections. The epidermis is composed of cells with a thickened outer wall, and within this are several layers of loose parenchyma. In the sections examined there were three vascular bundles, but whether this number is constant cannot now be stated. The bundles were of the usual collateral type with a few annular and spiral tracheids in the xylem. These bundles were separated from the central cylinder of conducting-tissue by

several layers of more compact parenchyma than that lying between the bundles and the epidermis.

The ovule fills the ovarian cavity almost completely, and there is no development of conducting papillae such as occur in *Naias*, either at the top of the ovary where the conducting tissue of the style terminates, or at the base of the funiculus near the micropyle. This may be accounted for by the fact that a pollen-tube which has reached the ovary can hardly fail to reach the micropyle, as its course along the wall would almost certainly bring it to the opening of the ovule.

The Embryo-Sac.

Unfortunately, not a sufficient number of specimens of the earlier stages of the embryo-sac were found to make it certain just how uniform the course of development is. With few exceptions there was nothing to indicate any marked departure from the ordinary type. In the earliest stage in which the archesporial cells could be recognized with certainty, there were two cells, evidently the product of the division of a primary hypodermal cell (Fig. 19). The outer of them was the larger, and is probably to be considered the real archesporium, and from it is apparently next cut off the primary tapetal cell (Fig. 18, *t*). There is thus formed a row of three cells in the axis of the nucellus. The form of the primary tapetal cell was quite different in different specimens examined (Figs. 18, 20), but in all cases it undergoes repeated divisions so that the sporogenous cell becomes more deeply sunk in the nucellus (Fig. 22).

The further history of the sporogenous cell must also be left somewhat incomplete, owing to the small number of satisfactory preparations of the next stage. It is extremely unlikely that the primary sporogenous cell ever develops at once into the embryo-sac, although such a form as that shown in Fig. 21 might possibly be so interpreted. In somewhat later stages (Fig. 22) there were found two or three cells derived from transverse divisions of the primary sporo-

genous cell, one of which by its subsequent growth destroys the others, and becomes the embryo-sac. In the case figured the lower cell already shows signs of disintegration, while in the upper one it is difficult to say whether we have the young embryo-sac showing the first nuclear division, or what seems more likely, from a comparison with other more advanced stages, the second division of the sporogenous cell, with a suppression of the cell-wall. In other cases where the young embryo-sac was found with two nuclei, it was much larger, and there were the remains of one, and in some cases of two sporogenous cells above it. Whether in any instances there are four complete sporogenous cells formed can only be determined by further investigations.

After it is once formed, the growth of the embryo-sac proceeds rapidly and the other sporogenous cells are destroyed. The youngest stages at which the embryo-sac could certainly be identified, already showed two nuclei. The cytoplasm did not fill the cell, but there was a large ventral vacuole. Near each end was a conspicuous but not very large nucleus, surrounded by a mass of granular protoplasm. The actual divisions of these nuclei were not seen, but there is no reason to suppose that they differ from other similar ones. As usual the four nuclei derived from each occupy either end of the embryo-sac. Those of the upper end are perhaps a little larger than those of the antipodal region, but the difference is very slight, and perhaps not constant. The nuclei are usually distinct with a single large nucleolus. The granular cytoplasm is now confined to a very thin layer at the sides of the embryo-sac, but is more abundant at the ends where the nuclei are situated. The remains of the sister-cell (or cells?) of the embryo-sac are still evident as a structureless mass lying above it (Figs. 24, 26).

There now begins the differentiation of the antipodal cells and egg-apparatus. The former, which are later very conspicuous, become invested with evident membranes, probably of cellulose, while the two synergidae and the egg soon become easily recognizable at the upper end. These are

distinct and bounded by a well-marked protoplasmic membrane. The two polar nuclei move toward the centre of the sac, where they finally fuse. There is usually no appreciable difference in the size or structure of the two, in which respect *Lilaea* agrees with *Naias* and *Zannichellia*. In his recent paper on the development of *Sagittaria variabilis*, Schaffner states[1] that the upper polar nucleus is by far the largest nucleus of the embryo-sac, and in the case figured in Fig. 24 the upper polar nucleus is slightly larger, but this is probably not constant.

The embryo-sac increases rapidly in size after the formation of the egg-apparatus and antipodal cells, and with this increase in size there is a change in the various nuclei, as well as marked growth in both the antipodal cells and the egg-apparatus. The former increase very much in size, and are usually arranged in a manner which often strongly suggests the arrangement of the cells of the egg-apparatus. The uppermost cell projects strongly into the cavity of the embryo-sac, and its nucleus becomes decidedly larger than those of the two lower cells (Fig. 28). In all of the cells the protoplasm is very granular, and there are often aggregations which stain strongly, and look almost like nuclei. The nuclei stain readily, and possess a single large nucleolus.

In the upper part of the embryo-sac the three nuclei of the egg-apparatus, which at first were nearly alike, soon show a decided difference in appearance. The two nearest the apex of the sac, the nuclei of the synergidae, increase very little in size, but remain clearly defined. The lower, or egg-nucleus, however, becomes many times larger than at first. The polar nuclei also increase very much in size, and the endosperm-nucleus resulting from the fusion (Fig. 27, *en.*) is the largest of all the nuclei in the embryo-sac. The synergidae, *s*, have a large vacuole, but the upper part, including the nucleus, is filled with granular protoplasm. The egg-cell extends some distance below the synergidae, and its granular

[1] Schaffner, The Life History of Sagittaria variabilis, Bot. Gazette, April, 1897.

protoplasm is more abundant, but contains numerous vacuoles. The nucleus is, as we have said, much larger than those of the synergidae, and contains more chromatin. The nucleolus is large, and in stained sections shows a vacuolated appearance. The large endosperm-nucleus is lenticular in form and has very little chromatin, but the very large nucleolus stains strongly and is much like that of the egg-cell. In the specimen figured there were two small bodies (*cen.*) lying near the nucleus which may possibly have been centrospheres; but they were not very conspicuous, and it is doubtful, at least, whether they can really be considered as such. They were not seen associated with the other nuclei in the embryo-sac, so that it must be considered questionable whether they were really centrospheres or only granules belonging to the cytoplasm.

While nearly all the embryo-sacs examined showed the normal structure just described, evidences of a deviation from this were seen in a few cases. The most marked was the one shown in Fig. 30. Unfortunately the structure of the lower part of the sac could not be clearly made out, as the series of sections was not complete. In the upper part of the sac, which was blunter than in the normal form, there was an irregular cellular mass, showing imperfect cell-walls. Eight nuclei could be certainly made out, but no trace of the definite egg-apparatus or other special structures usually found in the embryo-sac. Whether in the missing sections there were more nuclei than those seen, cannot be stated, but it is not impossible. Whether there were more than the normal number of nuclei, or not, the filling of the upper part of the sac with a cellular structure is a marked departure from the normal structure. Similar abnormal cases have been observed by the writer in *Naias flexilis*, *Zannichellia palustris*, and *Sparganium eurycarpum*; but otherwise, exceptions of this kind seem to have escaped observation.

POLLINATION.

The large stigmatic papillae and the conducting-tissue of the style, which is a continuation of the same epidermal tissue whose cells form the stigmatic papillae, have much the same appearance. The dense granular cytoplasm and large nuclei indicate that it is a secretory tissue, and with little question these cells are mainly concerned in forming the substances which serve to nourish the pollen-tube on its way to the ovary.

The ripe pollen-spore is nearly globular, and its finely reticulate exospore is ruptured by the pollen-tube (Fig. 14). The latter grows along the side of the papilla to which it is closely appressed. The growth of the pollen-tube through the conducting-tissue is not easily followed, and no especial study of this point was made, nor was the actual penetration of the pollen-tube into the ovule studied. There was nothing, however, to indicate anything peculiar in this respect.

One of the synergidae is probably destroyed by the growth of the pollen-tube, but one of these can often be detected even after the first division of the embryo (Fig. 29 *b*, *s*).

THE EMBRYO.

The development of the embryo was studied by Hieronymus, apparently with a good deal of care, and he gives numerous accurate figures of the different stages of development in his monograph. Unfortunately there is no account given in the text, nor is any explanation appended to the plates. On the whole his figures correspond closely to my own preparations.

After the egg becomes invested with its cellulose membrane as a result of fertilization, it elongates and divides, as most other Monocotyledons, by a transverse wall into two cells, a basal suspensor-cell, in contact with the upper end of the embryo-sac, and a terminal embryo-cell, which projects

into the cavity. The embryo-cell, as in *Naias* and *Zannichellia*, alone divides, the suspensor-cell remaining permanently undivided. Schaffner's recent observations on *Sagittaria* and *Alisma*[1] show this to be the case in these forms also, although Hanstein[2] supposed that the primary suspensor-cell underwent subsequent divisions.

The two cells arising from the first division of the egg in *Lilaea* are almost equal in size (Fig. 29), and much alike in the character of their cell-contents. With the elongation of the embryo, the free end becomes somewhat enlarged, and a transverse wall is formed in the embryo-cell (Fig. 31, *b*). The next division, at least in the few cases where this was seen, is in the terminal cell and is nearly vertical (Fig. 32). Following this is a transverse division in the middle cell, and next a further division, by a vertical wall, of each of the two terminal cells. The young embryo at this stage (Fig. 33) consists of six cells exclusive of the suspensor—the four terminal quadrants, only two of which show in the longitudinal section figured, and two cells between these and the suspensor. The latter has undergone no division, but there is a noticeable increase in the size of the nucleus which later becomes still more marked. In these early divisions of the embryo *Lilaea* agrees closely with *Zannichellia* and *Naias*, from which it differs mainly in the embryo being relatively shorter and the suspensor decidedly smaller. As in other similar embryos, the cells contain large vacuoles, the granular cytoplasm being principally confined to the neighbourhood of the nucleus and the periphery of the cell.

There is no absolute uniformity in the next divisions. It not infrequently happens that the first wall in the terminal cell is oblique, and it is possible that sometimes the second vertical walls may be suppressed. This seems to have been the case in the embryo shown in Fig. 36. Probably the next wall to form, following the quadrant-division in the terminal cell, is in ordinary cases a median vertical wall in the cell

[1] Schaffner, The Embryo-Sac of Alisma Plantago, Bot. Gazette, March, 1896.
[2] Sachs, Text-book of Botany, 1882, p. 589.

immediately below (Fig. 33), and this is later followed by a quadrant-wall in the same cell. The cell next above the suspensor divides by a varying number of transverse walls into a short row of cells, the uppermost of which undergo quadrant-divisions by vertical walls, much like those described in the cell next the terminal quadrants. The lowermost of the series of cells above the suspensor remains for some time, at least, undivided, but may finally undergo division by vertical walls.

The suspensor-cell may finally become much enlarged, but this takes place at a later period than is usual in *Naias* or *Zannichellia*; as in them, the nucleus finally reaches a very large size (Fig. 39).

The later divisions in the terminal cells vary a good deal, and the first division in each quadrant-cell may be either approximately vertical or longitudinal (Figs. 35, 37, 38). The second divisions are also more or less variable, but the result of the early divisions is usually the formation of a central group of four cells surrounded by a single layer of peripheral ones. The four inner cells form the primary group of plerome-cells for the cotyledon; while the outer ones, by subsequent periclinal divisions, develop the dermatogen and periblem.

While these divisions have been taking place in the terminal group of cells, a similar separation of a central group of plerome-cells also occurs in the segments lying just below the terminal group (Figs. 35, 37); and later, similar but less regular divisions occur in some or all of the basal segments. The number of these basal segments is usually three, and, as a rule, all of them sooner or later show vertical divisions; but transverse divisions are only found at a later period of development, and then only sparingly, so that the limits of the original segments may be made out for a long time (Fig. 39). Finally the limits become indistinguishable, and it is difficult to tell exactly how far the primary segments contribute to the different members of the older embryo. In this respect *Lilaea* differs from *Naias* and *Zannichellia*, where the relation

of the members of the embryo to the young segments is quite evident.

Owing to the formation of vertical walls in all of the lower segments, there is usually no secondary suspensor, such as usually occurs in other forms, but the enlarged vesicular primary suspensor-cell is in direct contact with the basal cells of the embryo (Fig. 43).

The whole of the cotyledon, and possibly also the stem-apex, is derived from the terminal segment of the young embryo; but owing to the late period at which the stem-apex is first recognizable, it is impossible to decide positively whether, as in *Naias*, it originates from the second segment, i. e. the cell immediately below the terminal quadrant-cells, or whether, as in *Zannichellia*, it is the product of the terminal quadrants (Figs. 40, 41). The cell-divisions in the terminal region proceed with great rapidity, while in the basal segments growth proceeds much more slowly. The embryo in consequence becomes pear-shaped, the lower narrow portion being the product of the basal segments, while the enlarged upper part is derived entirely from the terminal segment.

Fig. 39 shows a nearly median longitudinal section of an embryo where the primary tissue-systems are clearly defined, but the external differentiation is not yet indicated. The central plerome-strand is well marked, and shows in longitudinal section two rows of cells, separated from the dermatogen by the periblem, which is for the most part composed of two layers.

In an older stage (Fig. 40) the plerome-cells have undergone longitudinal divisions, and the periblem, especially in the cotyledon, has become very much more massive, owing to cell-divisions in all directions. Cross-sections of the young embryo are oval in outline (Fig. 44), and in the middle region (*c*) show a somewhat evident differentiation of the primary tissues, which is not clear in the basal region (*a*, *b*).

The first evidence of external differentiation is a slight depression on one side of the embryo, near the base. This marks the position of the stem-apex: but it is difficult to

tell whether it is derived from cells originating from the terminal segment of the embryo, or from the segment immediately below, as at this time the limits of the original segments can no longer be recognized with certainty. From its strictly lateral origin, however, and a comparison with other forms where the origin of the stem-apex is undoubtedly from the second segment, it is probable that in *Lilaea* the terminal segment gives rise to the cotyledon only, and that the stem is the product of the next segment.

The rapid increase in size in the embryo which now takes place is mainly due to the growth of the cotyledon, while the lower part of the embryo remains short. In the cotyledon the plerome-strand is easily seen (Fig. 41), but it is much less evident in the basal part of the embryo. The epidermis is well defined in all parts of the embryo.

In cross-sections of the older embryo made through the region of the stem-apex, the latter is seen lying in a shallow indentation formed by the base of the cotyledon, whose margins are beginning to form the sheath which later completely encloses it.

The point in which the embryo of *Lilaea* differs most markedly from that of other Monocotyledons which have been examined, is the origin of the primary root. This, instead of lying with the apex in direct contact with the suspensor, is decidedly lateral in position. In the earlier stages of the embryo it is impossible to make out clearly the relation of the tissues of the root to the other parts of the embryo. As soon as the root can be recognized as such, its axis is almost coincident with that of the stem (Figs. 41–43), and forms a marked angle with that of the cotyledon. It was not possible to decide positively, from the sections which were examined, what was the exact origin of the different root-tissues. The plerome is evidently continuous with the original axial plerome-cylinder of the young embryo, and is probably derived from the central cells of the second, and perhaps the third segment; i. e. it is derived, in part at least, from the same segment as the stem. In the older embryo

(Fig. 43) the broad plerome-cylinder of the root is conspicuous, and the other tissues at the apex of the root begin to show the arrangement found in the fully developed apex. At this stage in the specimens examined, no single initial cell could be made out for the plerome, whose apex was covered by a single layer of periblem-cells which back of the apex become divided by periclinal walls. Outside were two layers of cells, apparently formed by a periclinal division of the original dermatogen, and these give rise to the root-cap.

In the root of the full-grown embryo, the arrangement of the tissues is exactly like that of the roots of the adult plant. The plerome-cylinder shows about five rows of cells in longitudinal section, the central row being the largest, and probably later forming a central vessel. A single cell, somewhat larger than its neighbours, was seen at the apex of the plerome, and may possibly be a single initial, but this point needs further examination. A single layer of cells lies between the plerome and the root-cap, and this group of initials, by the periclinal division of its segments, gives rise to the epidermis and cortex of the root. The inner of the two layers of cells derived from the primary dermatogen (see Fig. 43) becomes the calyptrogen, and from it arise all the later layers of the root-cap.

As the embryo approaches maturity, a second leaf, much like the cotyledon, is developed opposite it, and later a third one at the base of the cotyledon[1]. In this condition the stem-axis has assumed a nearly vertical position, and with this displacement of the stem-apex there is a corresponding change in the position of the primary root, which comes to lie in nearly the same plane as the cotyledon. Hieronymus does not figure any intermediate conditions between quite young stages and the mature embryo, and to judge from his figures (nothing is given on the subject in the text of his memoir) he apparently supposed that the origin of the root was terminal, as in other Monocotyledons.

[1] See Hieronymus, Pl. IV, Fig. 42.

The Endosperm.

The formation of the endosperm begins shortly after fertilization. The primary endosperm-nucleus divides in the upper part of the embryo-sac, and the derivative nuclei distribute themselves in the layer of protoplasm lining its wall. The number of nuclei is large, and the protoplasmic layer becomes a good deal thickened, but no cell-divisions were seen in the endosperm. The nuclei are distinct, each with a single conspicuous nucleolus (Fig. 44, *a*), and vary a good deal in size. The embryo finally fills the embryo-sac completely, and in the mature seed there is no trace of the endosperm.

Summary.

1. The flowers of *Lilaea* are of strictly terminal origin, both anther and ovule being formed directly from the transformed apex of the shoot.

2. The sporogenous tissue of the stamen is not hypodermal in its origin, but arises from the plerome, as in *Naias* and *Zannichellia*.

3. The ripe pollen-spore has two cells. The generative nucleus remains undivided in the ripe spore.

4. The archesporium of the ovule is hypodermal, and a tapetal cell is cut off from it.

5. The primary sporogenous cell of the ovule divides usually into three, of which the middle one becomes the embryo-sac.

6. The embryo-sac usually develops in the manner typical of the Angiosperms, but there may be a suppression of a definite egg-apparatus, and a formation of cellular tissue in the upper part of the embryo-sac before fertilization. This is probably accompanied by an increase in the number of nuclei, such as has been observed in other low Monocotyledons.

7. The first division of the embryo is the typical one into

two cells, a basal suspensor-cell which remains permanently undivided, and a terminal embryo-cell.

8. The cotyledon is derived entirely from the terminal one of the primary segments into which the embryo-cell first divides.

9. The stem probably always originates from the second embryonal segment, but this point is still somewhat doubtful. Its position is strongly lateral.

10. The root is of lateral origin, in this respect differing from other Monocotyledons which have been studied.

11. The root of the mature embryo is entirely like that of the older plant.

CONCLUSIONS.

From the study made of the development of the flower and embryo of *Lilaea*, it is clear that it shows resemblances to the other low Monocotyledons which have been studied. The apical origin of the sporangia is very much like that of *Naias* and *Zannichellia*, and it is quite probable that this will be found to be the case in other low types, such as *Sparganium* and the Potamogetonaceae; but further investigations are necessary to determine this.

The origin of the stamen and carpel in cases where they occur together, from the dichotomy of a common primordium, makes it probable that the resulting complex should be considered as a secondary inflorescence composed of two flowers, rather than as an hermaphrodite flower.

While the development of the embryo-sac is normally that of the typical Angiosperms, the occurrence of exceptional cases with a probable multiplication of the nuclei and the development of cellular tissue before fertilization is significant, especially in view of the similar phenomena in *Naias*, *Zannichellia*, and *Sparganium*, and suggests a possible case of reversion to a more primitive condition.

The development of the embryo itself is most remarkable for the peculiar lateral origin of the root, which is quite

different from that in the typical Monocotyledons. Just what the significance of this is, is hard to determine. This lateral position is rather suggestive of the root in *Isoetes*, and possibly the basal segments of the embryo with the suspensor might be interpreted as equivalent to the foot in the embryo of the Pteridophytes. A study of the embryo in other simple Monocotyledons may yield some further information upon this point.

While it must be admitted that these investigations do not throw much light upon the question of the origin of the simpler Monocotyledons from pteridophytic ancestors, such as *Isoetes*, it may also be said that there is no further evidence for the view commonly held that they are degenerate forms, descended from more specialized ancestors. There is certainly no evidence that the flowers are derived from any type found among the higher Monocotyledons, and the writer is strongly inclined to believe that the simplicity of the flowers is really primitive. It is hoped that further investigations, which it is proposed to make, may possibly help to elucidate this very interesting subject.

EXPLANATION OF FIGURES IN PLATES I, II, AND III.

Illustrating Professor Campbell's Paper on *Lilaea subulata*.

PLATE I.

Fig. 1. Median longitudinal section of a young plant; × 40; *l*, *l*, leaves; ♀, female; ♂, male flowers; *sq*., squamulae intravaginales; *i*, lacunae in the leaves.

Fig. 2. A similar section of a younger plant with the primary axis transformed into the first inflorescence, ×; lettering as in Fig. 1.

Fig. 3. Median section through a very young floral complex from the middle region of the inflorescence; × 600 (about): *l*, the subtending bract; ♂, male; ♀, female flower.

Fig. 4. A similar section of an older floral complex; × 100.

Fig. 5. Longitudinal section of the young stamen, showing the arrangement of the primary tissues; × 350.

Fig. 6. A similar section of an older stamen; × 100; *l*, the subtending bract.

Fig. 7. Cross-section of a young stamen, showing the four loculi; *s*, sterile lobe; × 350.

Fig. 8. A single loculus from another of about the same age as that shown in Fig. 6; × 600. The limits of the plerome are indicated by a heavy line.

Fig. 9. Cross-section of a loculus showing the young sporogenous tissue; ×600. The sporogenous cells have the nuclei shown.

Fig. 10. Longitudinal section of the anther shortly before the isolation of the sporogenous cells; × 600; *c*, *d*, the outer tapetal cells; *t*, the cells of the inner tapetum.

Fig. 11. Young spore-tetrad imbedded in the nucleated protoplasm derived from the disintegrated tapetal and sterile sporogenous cells.

Fig. 12. Section of a nearly ripe pollen-spore, with the generative cell, *g*, and the nucleus, *v*, of the large vegetative cell; × 600.

Fig. 13. Cross-section of the wall of the ripe anther; × 600.

Fig. 14. Germinating pollen-spore upon one of the stigmatic papillae, *p*; ×600.

Fig. 15. Longitudinal section of a very young basal (long-styled) female flower; × 350; *o*, the ovule.

Fig. 16. The ovule from a somewhat older flower; *v*, the apex; × 600.

Fig. 17. Longitudinal section through a very young short-styled flower; *car*. the carpel; *o*, the ovule; × 600.

Fig. 18. Longitudinal section of an ovule after the differentiation of the first integument, *in*.; *t*., the primary tapetal cell; × 600.

PLATE II.

Fig. 19. Longitudinal section of young short-styled flower; × 600; *o*, ovule; *car*., carpel.

Fig. 20. Longitudinal section of the young ovule, showing the beginning of the first integument, *in*.; × 600.

Fig. 21. An older ovule with the tapetum (*t.*) already divided; in^1, first, in^2, second integument; × 600.

Fig. 22. A still older ovule. The lower cell of the axial row is becoming disorganized, and the one above it has two nuclei, but no division-wall has formed between the latter.

Fig. 23. The nucellus of an older ovule, showing the young embryo-sac with two nuclei; the section is cut somewhat obliquely; *m*, the remains of the upper archesporial cell.

Fig. 24. An older embryo-sac with eight nuclei; *p*, p^1, the polar nuclei. The egg-nucleus lies immediately below the upper polar nucleus.

Fig. 25. Longitudinal section of a flower with style of medium length, showing the stigmatic papillae, *st.*; × 100.

Fig. 26. Embryo-sac with egg-apparatus and antipodal cells fully formed, but the polar nuclei not yet united; only two antipodal cells show in the section; × about 600.

Fig. 27. Upper part of a fully developed embryo-sac; *en.*, the endosperm-nucleus; × 650.

Fig. 28. The antipodal end of the fully developed embryo-sac; × 650.

Fig. 29. Two sections of an embryo-sac with a two-celled embryo; *s*, the remains of one of the synergidae; × 300.

Fig. 30. Upper part of an abnormal embryo-sac. There were eight nuclei, two of which do not show in this section.

Figs. 31–35. Successive stages in the development of the embryo, in longitudinal section; × 600. The order of the transverse divisions is indicated by the lettering. The embryo shown in Fig. 33 had the terminal cell divided into four.

Fig. 36. A young embryo in which the second wall (2) in the terminal cell was oblique, instead of being formed at right angles to the first one.

Figs. 37, 38. Two sections of an older embryo.

PLATE III.

Fig. 39. A somewhat advanced embryo, seen in median section, showing the enlarged suspensor-cell with its nucleus. The limits of the first transverse walls are still visible.

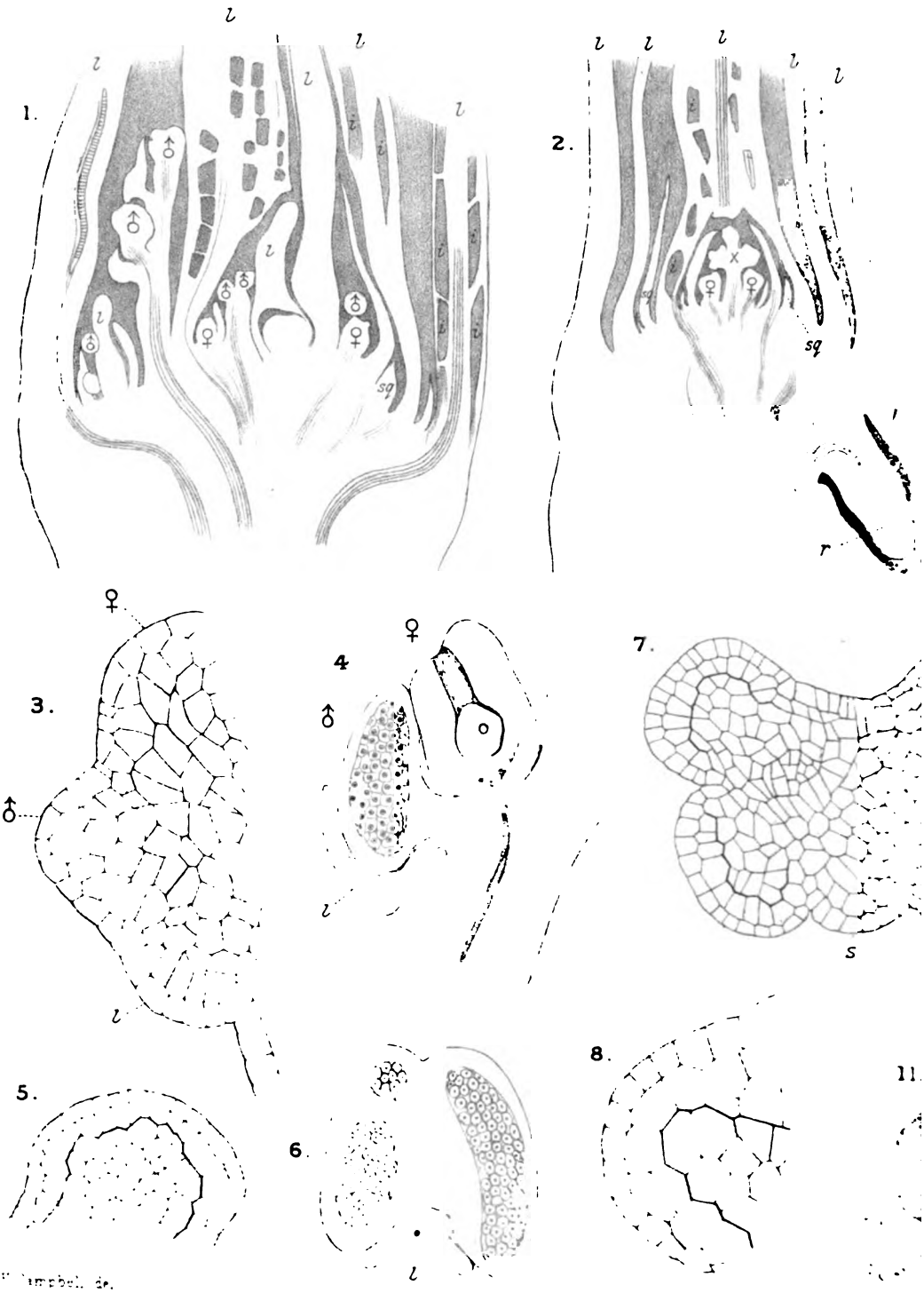
Fig. 40. A similar section of an older embryo, showing the first trace of the stem-apex, *st.*, and the root, *r*.

Fig. 41. A still older embryo, showing the strongly lateral position of the primary root, *r.*; *st.*, stem-apex; *cot.*, cotyledon.

Fig. 42. Section of an embryo from a nearly ripe seed; × 100.

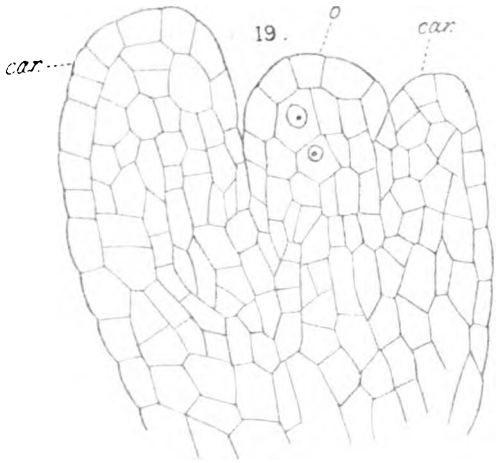
Fig. 43. The basal part of a similar embryo; × 350.

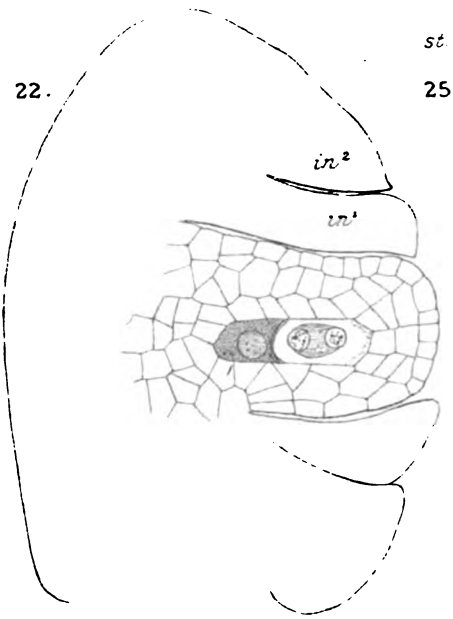
Fig. 44. Three transverse sections of a young embryo; × 350; *a* is a section just above the suspensor, and shows several of the endosperm-nuclei.

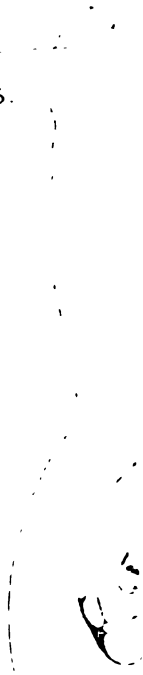


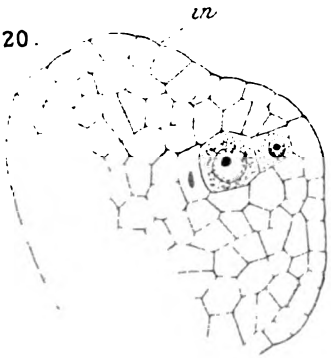
CAMPBELL. — LILAEA SUBULATA.

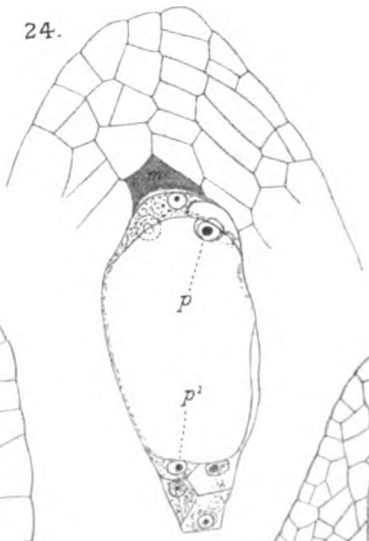
9
14.
p.
15.
o
car
o
16.
13
17.
10
18
12

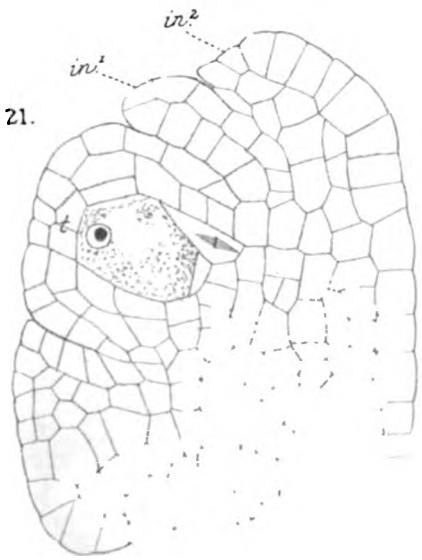






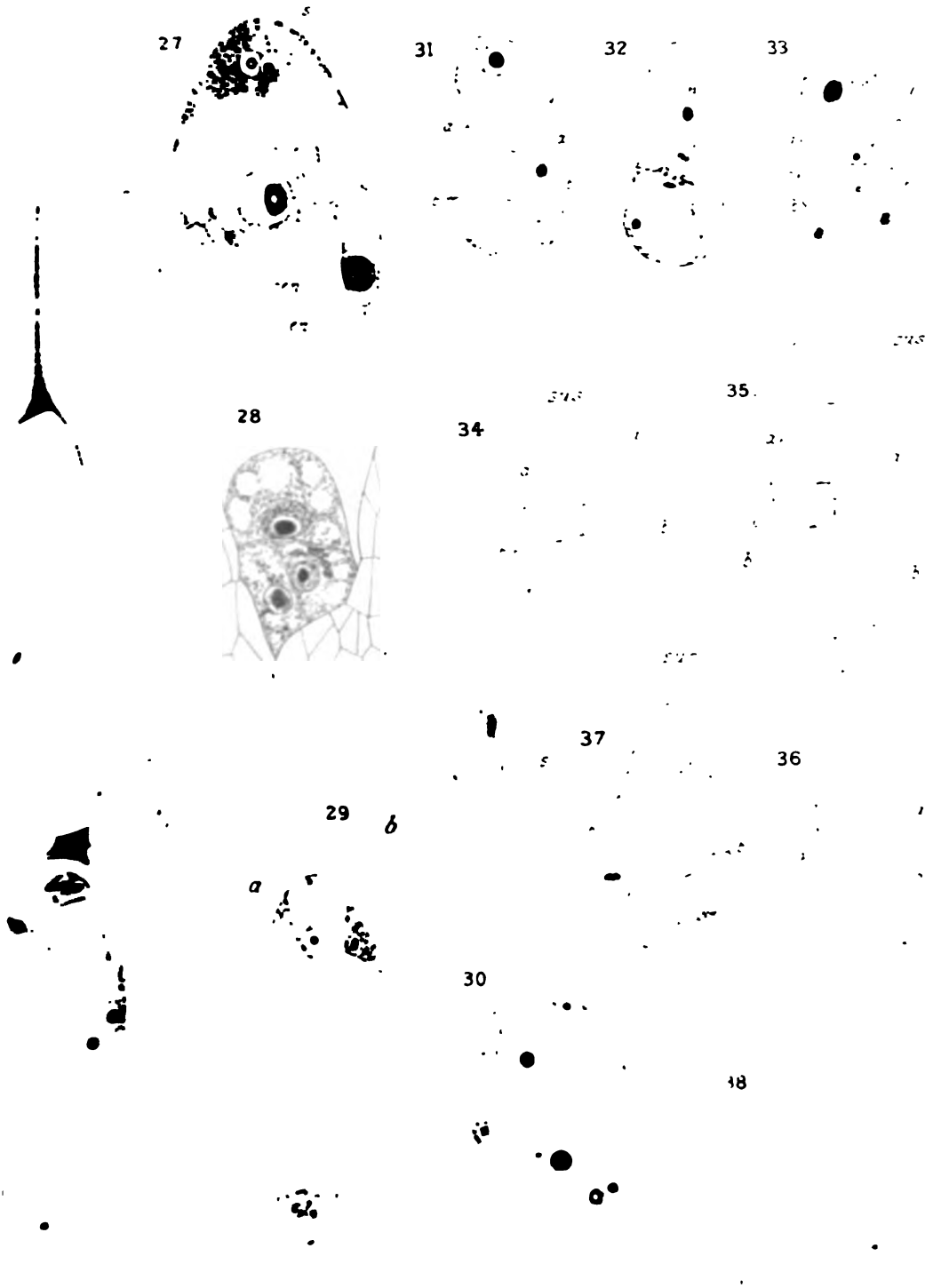


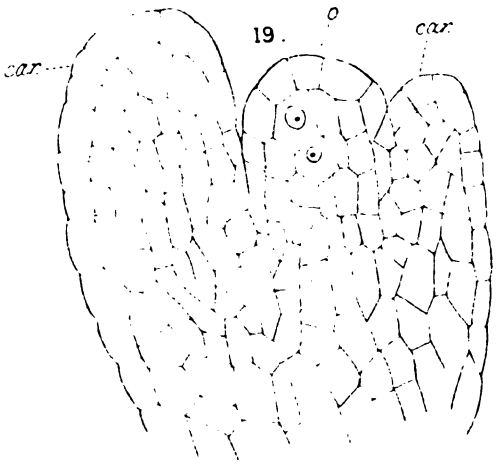


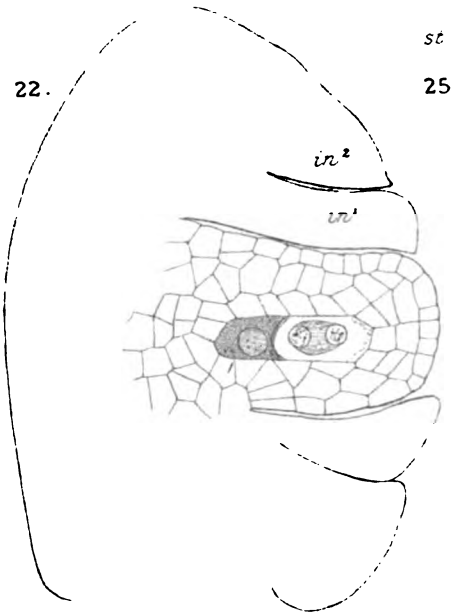


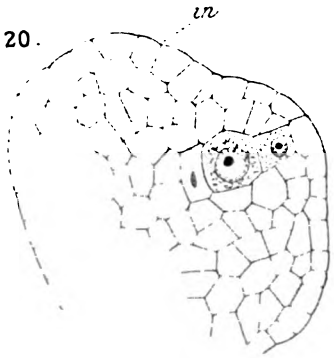
D H Campbell del.

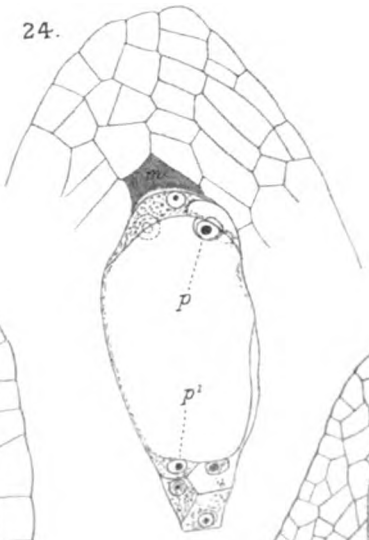
CAMPBELL.— LILAEA SUBULATA.

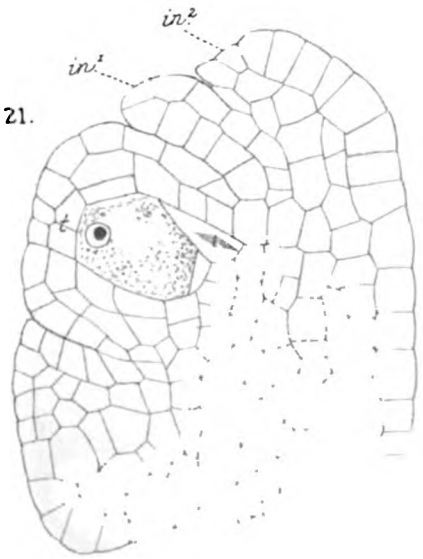

27
31
32
33
28
34
35
37
36
29
b
a
30
38

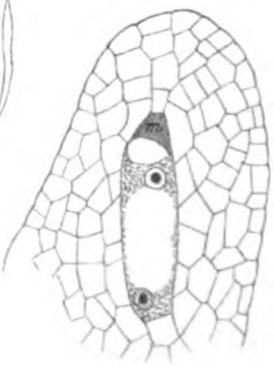






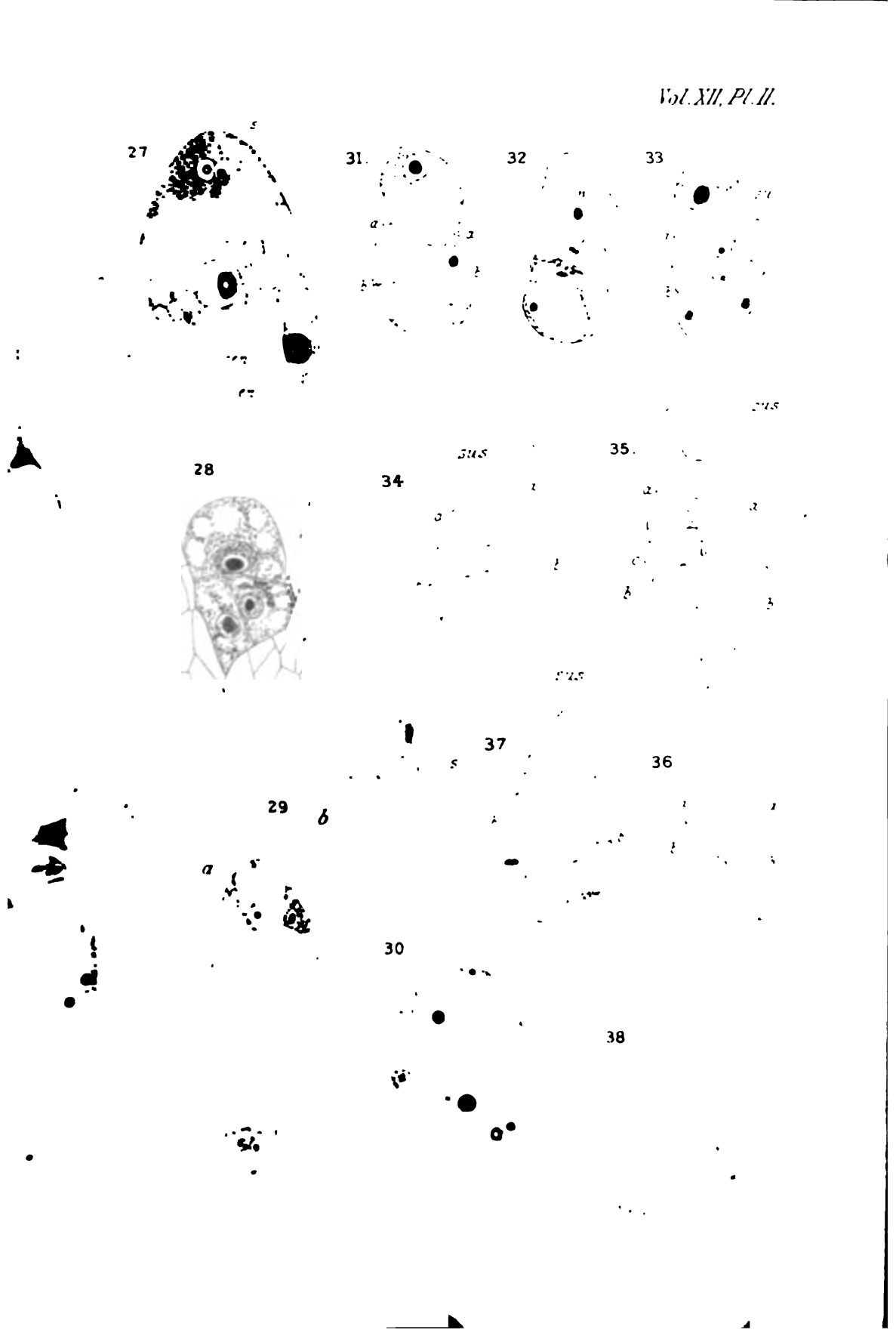


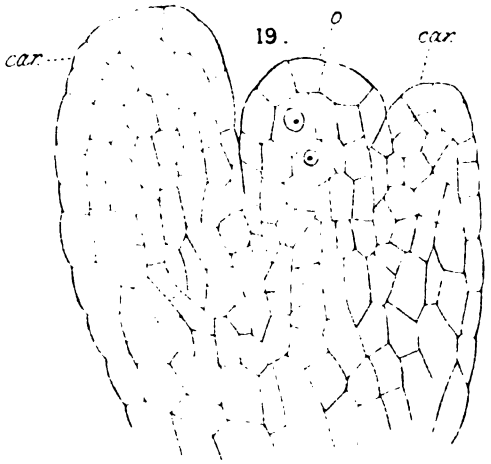


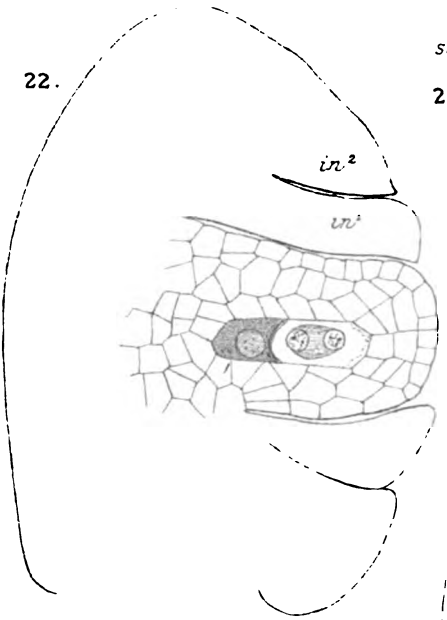


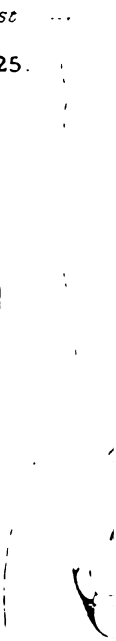
D H Campbell del.

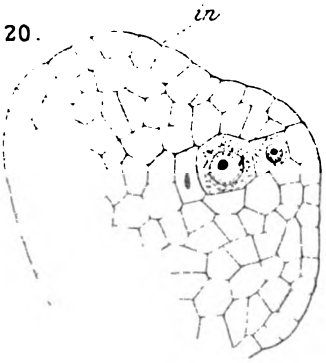
CAMPBELL.— LILAEA SUBULATA.

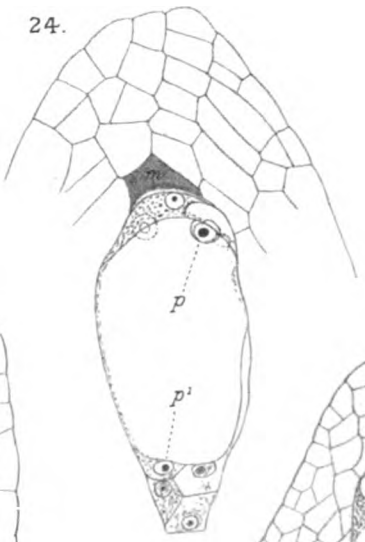

27
31.
32
33
28
34
35.
37
36
29
b
a
30
38

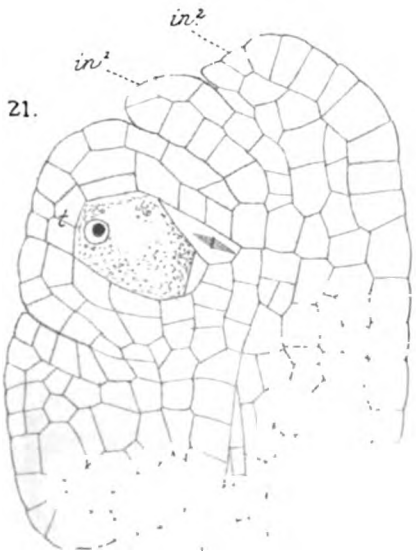


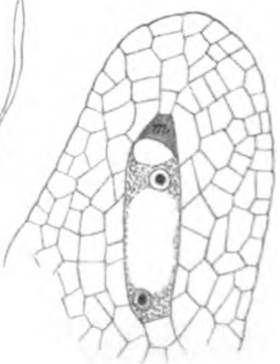






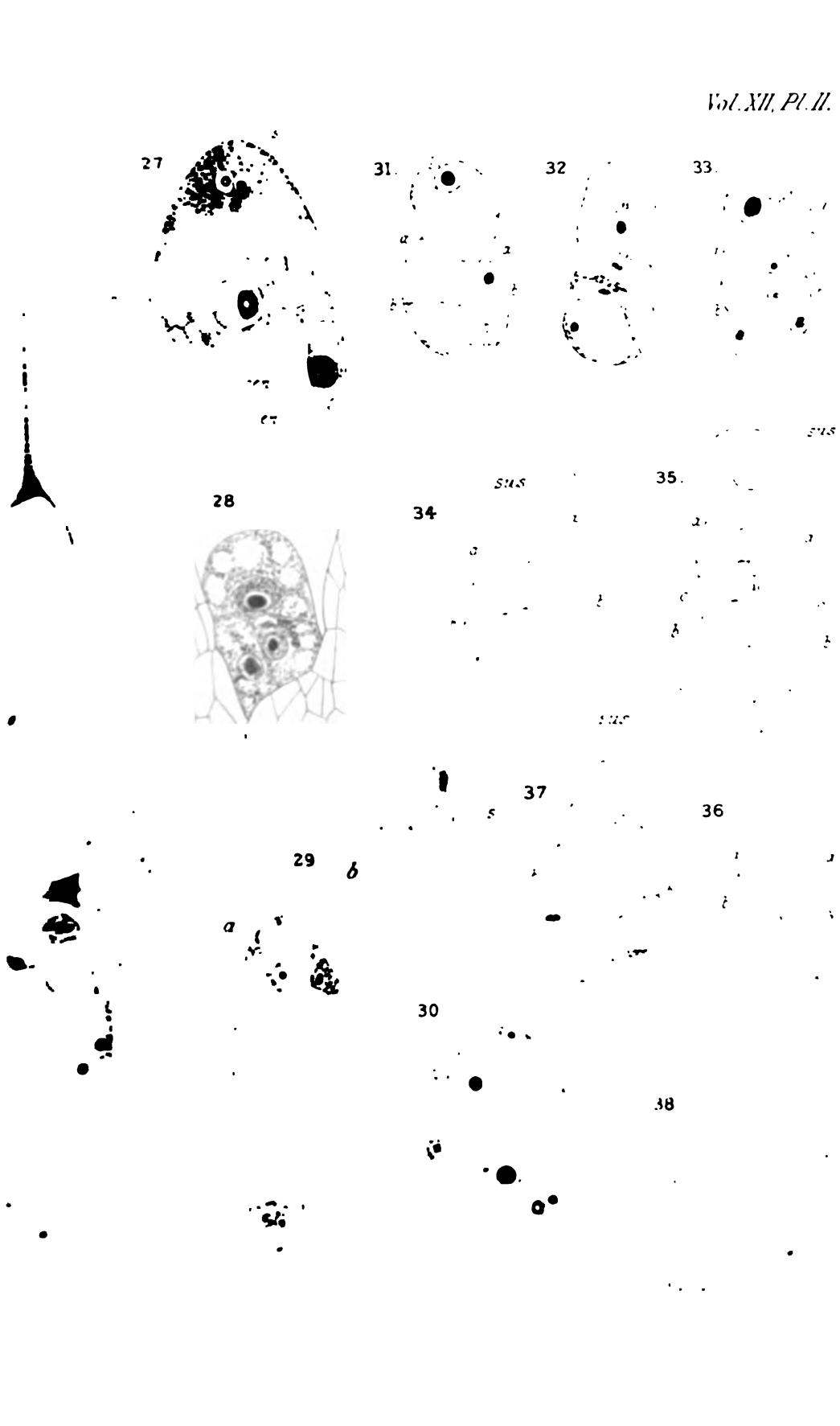






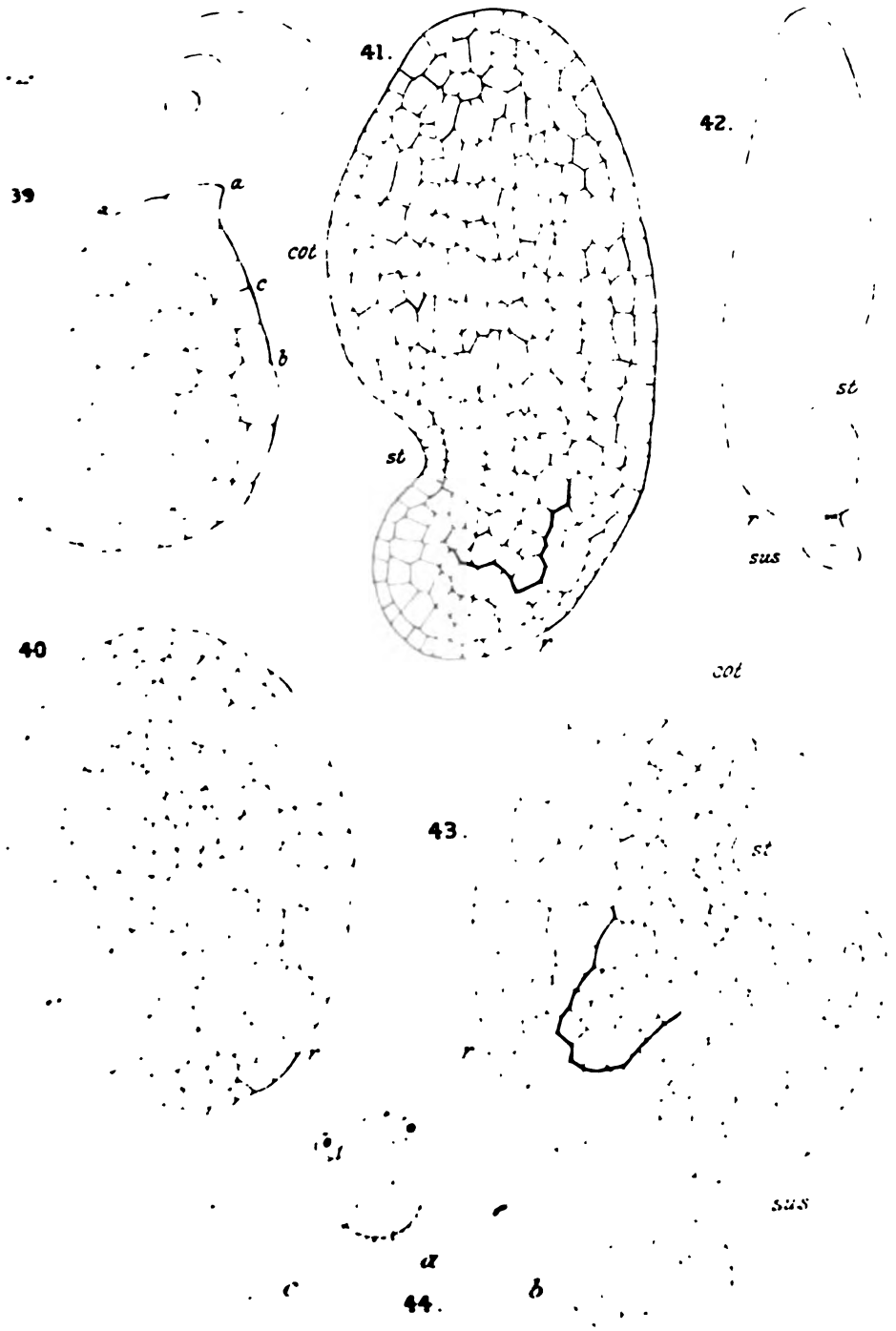
D H Campbell del.

CAMPBELL. — LILAEA SUBULATA.


27
31
32
33
28
34
35
37
36
29
b
a
30
38

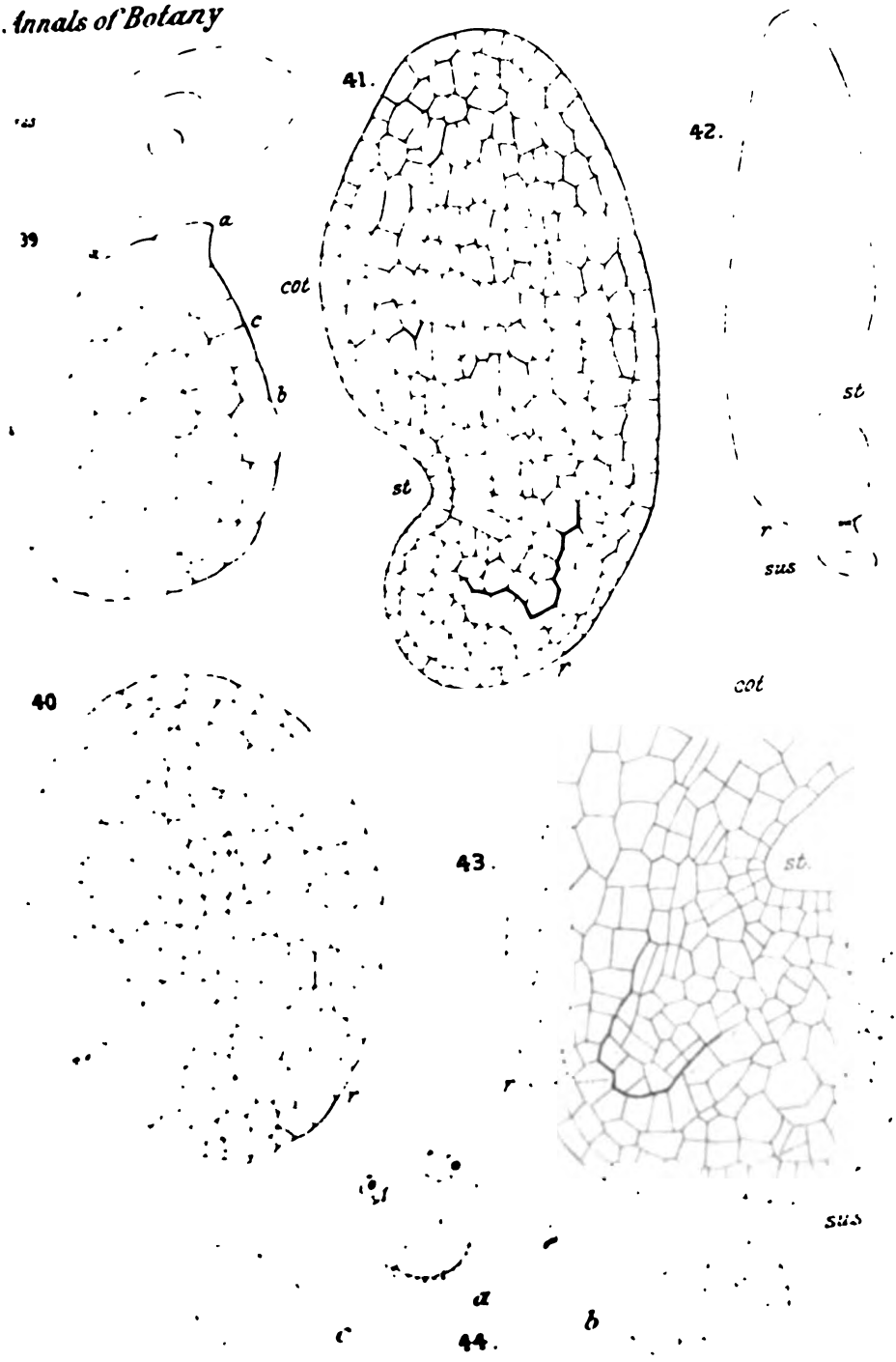
Campbell del. University Press, Oxford

CAMPBELL — LILAEA SUBULATA



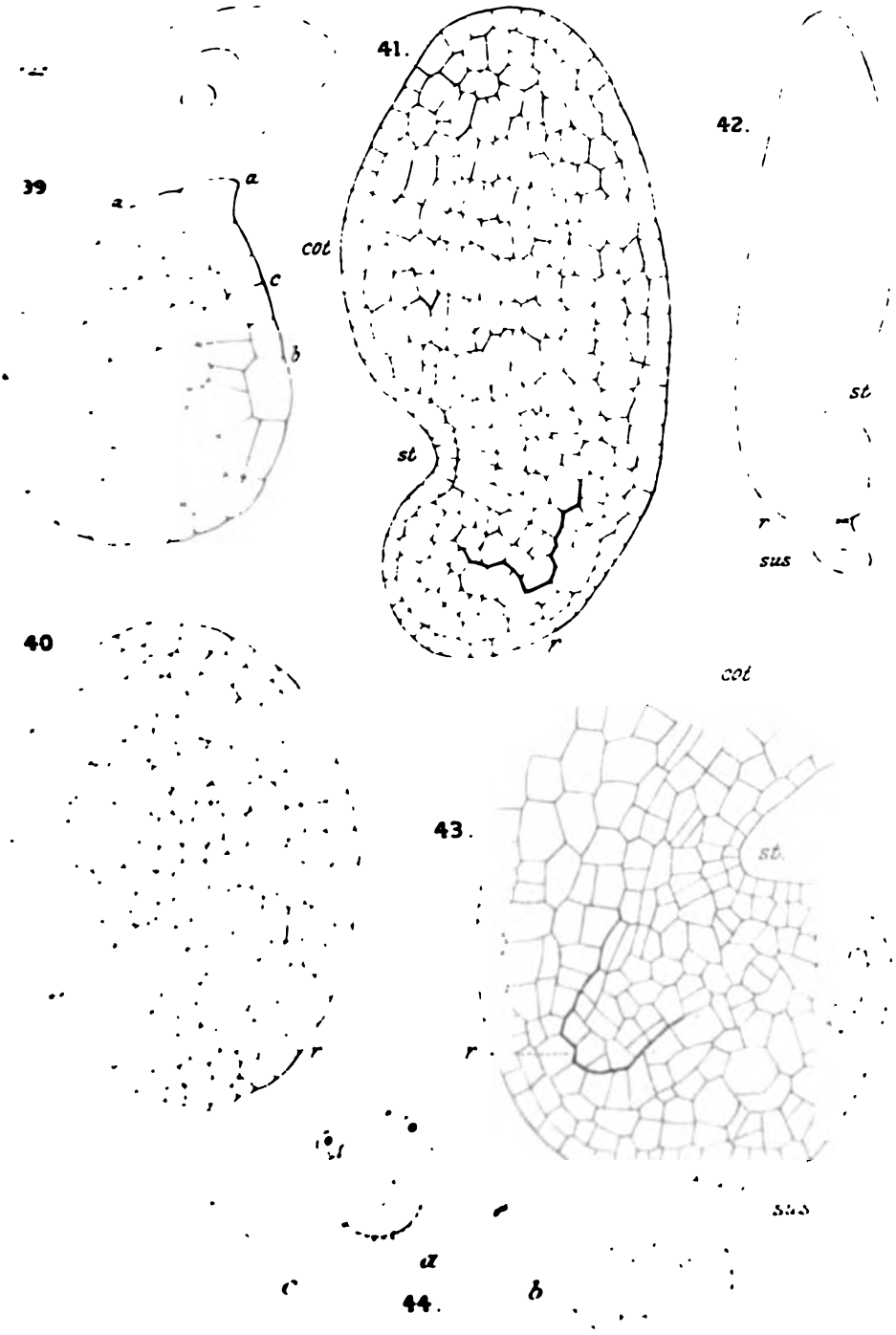
CAMPBELL — LILAEA SUBULATA

CAMPBELL — LILAEA SUBULATA



Digitized by [illegible]

CAMPBELL — LILAEA SUBULATA



CAMPBELL — LILAEA SUBULATA

Observations on the Conjugatae.

BY

W. WEST, F.L.S., & G. S. WEST, A.R.C.S.

With Plates IV and V.

DURING a prolonged study of Freshwater Algae from all parts of the world, many more or less interesting observations concerning the Conjugatae have accumulated: in this paper we propose to set forth some of them, together with certain conclusions derived therefrom. This group of Algae has been extensively studied by many previous botanists, amongst whom particular mention may be made of De Bary, Wittrock, Nordstedt, Lagerheim, Klebs, Bennett, and others; and we have here attempted to correlate with each other and with our own observations, a few of the facts described by these several observers, and from this to ascertain, as nearly as possible, the relationship existing between the various members of the group.

We classify the Conjugatae into the three following families: ZYGNEMACEAE, TEMNOGAMETACEAE, and DESMIDIACEAE, and in this paper we think it advisable to deal with them separately.

Many authors regard the Mesocarpeae as a separate family, owing to the peculiar formation of the spores; but we think

[Annals of Botany, Vol. XII. No. XLV. March, 1898.]

it is better regarded as a sub-family of the Zygnemaceae, as *Pyxispora* has the same method of formation of its spores, although the chromatophores are similar to those of *Zygnema*.

These plants may occur as solitary cells, or they may be filaments which at some or all periods of their existence more or less easily dissociate into the separate cells of which they are composed. The genus *Gonatozygon* may be taken as an illustration; it is sometimes found in long filaments of about thirty or more cells; but on being subjected to the least disturbing influences these filaments break up, and in some species of the genus the filamentous condition is rarely attained. There is a tendency in many of the small species of *Cosmarium*, very noticeable in *C. moniliforme* and *C. Regnellii*, to assume a filamentous condition, and this may have induced Rabenhorst[1] to place *C. pygmaeum* under *Sphaerozosma*. We have also noticed this tendency in *Euastrum binale* (cf. Fig. 38). In *Micrasterias foliacea*, a representative of a genus the species of which normally occur as solitary plants, this filamentous condition has been attained by a remarkable degree of specialization of the polar lobes of the semi-cells, which possess an arrangement of apical teeth which interlock so firmly with those of the adjoining cell, that the connexion is too rigid to allow of hardly any flexibility in the filaments. A filamentous condition of the genus *Mesotaenium* (which is generally unicellular) is found in the Arctic plant named by Berggren *Ancylonema Nordenskioldii*. The filaments of *Hyalotheca* dissociate into separate cells just prior to conjugation, and the dissociated cells remain imbedded in a mucus derived from that which surrounded the original filaments. Conjugation is very soon general throughout the mass, as can readily be seen in a conjugating example of *H. dissiliens*. The fragmenting of the old filaments into individual cells is well known as a method of reproduction in some genera of Zygnemaceae. It can therefore be considered that a strictly filamentous condition is of no essential importance to the life of the Conjugatae.

[1] Flor. Europ. Algar. III, p. 150.

All Conjugates are surrounded by a definite mucilaginous envelope. In the great majority this covering is very thin, but in others it is profusely developed, e.g. *Zygnema anomalum*, *Hyalotheca mucosa*, *Staurastrum tumidum*, *S. longispinum*, &c.; and even in those species in which it is normally almost absent it is occasionally developed to a large extent. We have seen very extensive mucilaginous envelopes round *Closterium Lunula*, *Penium Libellula*, and *Cosmarium ovale*, species which are normally destitute of such extensive investments. No doubt this mucus serves in many cases as a means of attachment, as we have seen as many as a dozen specimens of *Staurastrum tumidum* attached to one leaf of *Utricularia minor*, and in the case of many of the species which occur on dripping rocks, this mucus is absolutely necessary for the purpose of attachment; but an equally important use is probably that of protection from epiphytes and parasites (Chytridiaceae, &c.). With regard to the nature of this gelatinous investment, it must be considered either as a secretion or a mucous condition of the outer layers of the cell-membranes. Klebs regards it as quite independent of the substance of the cell-wall; and as the cell-membrane of most Desmids is perforated by a large number of minute pores —excessively minute in some and not visible although perhaps present in others—one would be inclined to regard this mucous envelope as a secretion. But in some species of *Zygnema* it seems to us to be partly if not entirely due to the diffluent outer layers of the cell-wall, and this may be also true for some Desmids. In an almost pure gelatinous gathering of *Cosmarium cymatopleurum*, var. *tyrolicum*, many of the specimens were seen casting the outer coats of the cell-membrane, and in some cases many such successive coats could be seen round each individual gradually fading into the mass of jelly in which the plants were imbedded. This gathering was from the vertical face of a dripping rock. We have also noticed *Cosmarium pyramidatum*, when imbedded in a gelatinous mass of Desmids, casting its outer cell-membrane in a similar manner.

That this mucus is often of a tough nature is proved by a consideration of the genus *Spondylosium*, in which the individuals are united into long filaments by a layer of mucus between the apposed ends of the cells; and that the connexion is by no means a weak one is shown when the filament is fractured, the cells more often breaking across the isthmus than coming apart at the apical attachment.

Organs for attachment are occasionally developed in young plants of *Spirogyra*[1] (Fig. 20) and *Mougeotia* (Fig. 16), but have not been noticed in any other genus of Conjugatae; they are special outgrowths (simple or branched) at the base of the shoot, and are homologous to those organs of attachments found amongst other Algae and usually termed *rhizoids*, but more recently known as *haptera*. We have also noticed them to be developed in *Spirogyra*, as a result of the modification of a conjugating-tube protruded by a cell some distance removed from those cells of the filament engaged in conjugation (Fig. 21).

Branching amongst members of the Conjugatae is abnormal and of somewhat rare occurrence. When present in the Zygnemaceae it is generally limited to lateral outgrowths consisting of a few cells; we have only noticed it in the genera *Zygnema*[2] and *Mougeotia* (Figs. 17–19). In the Desmidieae one semi-cell occasionally undergoes a partial lateral[3] or dichotomous[4] branching (Fig. 40).

The apical cells of filamentous Zygnemaceae are generally rounded at the free end, but they often become elongate and irregular.

The filamentous forms consist of but one series of cells, longitudinal septa rarely making their appearance; they have

[1] Borge, Ueber die Rhizoidenbildung einig. fadenförm. Chloroph., Upsala, Nya Tidnings Aktieb., Tr. 1894. Wolle, Freshw. Alg. U. S., Pl. CXLII, f. 7, 8.

[2] Cf. *Zygnema pachydermum*, West, Alg. from W. Indies, Journ. Linn. Soc. Bot., Vol. xxx, Pl. XIII, Figs. 12–15.

[3] Reinsch, Contrib. Alg. et Fung., T. xviii, f. 12 and 15.

[4] Jacobsen in Journ. de Botanique, Copenhague, 1874, t. viii, f. 31.

been noticed in an incomplete form in *Zygnema pachydermum*, var. *conferwoides*[1].

With regard to the effect of temperature on the Conjugatae, a paper by Alfred J. Ewart, entitled 'On Assimilatory Inhibition in Plants,' has recently appeared[2], in which the author states (p. 395) that 'freshwater Algae . . . are not very resistant to cold, all those examined being killed by being frozen. This statement we cannot agree with, as we have found them to be very resistant to cold, and as a large number of the plants belonging to the Alpine Algal flora are Conjugates we illustrate the matter by a few examples.

We have melted out of the ice from Mitcham Common, Surrey, excellent examples of *Spirogyra cataeniformis* in a state of conjugation, the vitality of which was in no way impaired. From Frizinghall, W. Yorks., we have also melted out of the ice hundreds of specimens of *Closterium Leibleinii*, which subsequently remained in a perfectly healthy and normally active condition (moving to that side of a vessel exposed to most light, just as we find all other species of Desmids to act), and in each of these cases the specimens examined had been frozen for over fourteen days. These facts alone disprove the generality of Mr. Ewart's statement; but let us now consider some still more convincing ones. Many of the upland tarns of Yorkshire, the Lake District, the Scotch Highlands, and other places, are situated at altitudes of over 2,000 feet, some of them being much higher, and the water in them is of a relatively low temperature even in summer. For many months in the winter these tarns are frozen, and the small ones often buried in deep snow drifts, although by the middle of summer they are fairly crowded with filamentous Algae, of which the most abundant forms are small species of *Mougeotia*. We have never yet seen these species of *Mougeotia* in conjugation from these altitudes[3],

[1] Cf. West, l.c., Pl. XIV, Fig. 5.

[2] Journ. Linn. Soc. Bot., Vol. xxxi, 1896, No. 217.

[3] We have examined sterile species of this genus, obtained at 3,000 feet in the Scotch Highlands and 6,500 feet in Switzerland.

and in ordinary seasons in all probability they never do conjugate. How, then, are they preserved throughout the winter? It must be by means of the survival of some of the plants (without the formation of spores) through the prolonged freezing they have to undergo, which is followed by their active division in the spring. We can also mention instances of this in the Desmidieae. There is a small peaty ditch in Eldwick, on the edge of Rombald's Moor, W. Yorks., in which countless numbers of *Micrasterias denticulata* have occurred in a perfectly pure state for very many years. We have examined this ditch carefully at all times of the year, and always find some specimens of this Desmid, even when it is frozen; but never once have we come across a single zygospore from this locality, although constant search for them has been made. Here, then, the perpetuation of the species must be dependent upon the survival of some of the ordinary vegetative plants through the winter; and we may mention that the locality is a bleak one, its altitude being near 1,000 feet, and the water is generally frozen for some weeks during the winter.

It is also worthy of note that in all the contributions to the Algal Flora of the Arctic regions yet published, the occurrence of the zygospores of Desmids has seldom been mentioned, though many species are recorded from places noted for their intense cold in winter, for instance, Greenland, Spitzbergen, Nova Zembla, and northern Siberia. We have a species of *Oscillatoria* from a valley in the Davos Platz district in Switzerland, collected by Mr. A. Howard in August, 1897, from a stream at 8,000 ft. elevation with the temperature of the water at 5°C. This is the summer condition, and the winter one may be easily imagined; we must therefore reject Mr. Ewart's statement that 'owing to their slight powers of resistance to cold, the temperatures to which they can be exposed without being permanently injured are necessarily relatively high.' Stationary masses of water, such as pools and small lakes, even at this altitude, attain during summer a comparatively warmer temperature than the streams; a lake

close to the above-mentioned locality, and at the same altitude, had a water-temperature of 20°C. In this lake fine examples of *Staurastrum Meriani* occurred, and it is evident that they must be capable of withstanding frost for a few months during the winter. We have found specimens of *Closterium striolatum* and *Cylindrocystis Brebissonii*[1] in material collected on the top of Green Hill, Clova Mts., at 2,700 ft., from water which was derived from melting snow close by, and which could not be more than 1° or 2°C. These specimens were collected by Mr. J. H. Burkill in May, 1897. We also point out the four following papers dealing entirely with snow-floras:—

S. Berggren: Alger från Grönlands inlandis (Öfvers. K. Vet.-Akad. Förh. 1871, No. 2).

V. B. Wittrock: Om snöns och isens flora, särskeldt i de arktiska trakterna (A. E. Nordenskiöld, Studier och forskningar föranleda af mina resor i höga norden), Stockholm, 1883.

G. Lagerheim: Bidrag till kännedomen om snöfloran i Luleå Lappmark (Botanisker Notiser, 1883, Heft 6), Lund, 1883.

G. Lagerheim: Die Schneeflora des Pichineha, ein Beitrag zur Kenntniss der Nivalen Algen und Pilze (Bericht. d. Deutsch. Botan. Gesellsch., Jahrg. 1892, Bd. x, Heft 8), Berlin, 1892.

In the same paper by Mr. Ewart we also find (p. 439) the following statement: 'It is well known that prolonged exposure to direct sunlight is fatal to . . . many Algae.' From our own experience we should at once say that nothing could be more beneficial to Freshwater Algae than prolonged exposure to direct sunlight, provided they remain under natural conditions.

Round the margins of the two ponds on Frensham Common, Surrey, there is a belt of very shallow water, which is the home of large numbers of Algae, and these plants on bright

[1] This species occurs in pure gelatinous masses (during early spring before Easter) on the peat at the extreme summit (2,346 feet) of Great Shunnor Fell in N. Yorks.

days are not unfrequently exposed to direct sunlight from almost the rising to the setting of the sun. We have noticed this shallow water become quite warm. What is the effect of this prolonged exposure to sunlight and the increase in the temperature of the water? It is certainly not a detrimental one, because there is an acceleration in the growth of the lower green and blue-green Algae [1], and the Conjugatae form zygospores much more abundantly than they otherwise would do: we could multiply instances indefinitely, but the following one will suffice.

From Vehar Lake, Parel, Bombay, we have examined the finest specimens of *Clathrocystis aeruginosa* we have yet seen, and these are exposed to direct sunlight every day for weeks; moreover the atmospheric (shade) temperature was 96° Fahr., and that of the water 87° Fahr. The material was collected for us in 1895 by Mr. S. Tomlinson, C.E., the Government Engineer to the Waterworks.

Yet Mr. Ewart would inform us that prolonged exposure to direct sunlight is fatal! It is so (as we well know) in the small vessels of the laboratory, but not in nature [2].

There are four methods of reproduction in the Conjugatae: by fragmentation of the filaments (asexual); rarely in some genera by resting-cells or cysts (asexual); by conjugation with formation of zygospores or carpospores; and by aplanospores (asexual). Temperature and climatic conditions affect reproduction only so far as to promote or prevent it; they have little effect on the method, although an increase of temperature considerably helps conjugation, and so far as we have observed, a higher altitude (which is usually accompanied by a lower temperature) favours the formation of 'cysts.'

During conjugation the activity of the filament is increased; even those cells which take no part in it show greater vigour.

[1] Specially noticeable were *Clathrocystis aeruginosa* and *Crucigenia rectangularis*, the latter with single families of 128 cells, the normal number being 16 or 32.

[2] The reader should consult the excellent work by Klebs entitled 'Bedingungen der Fortpflanzung bei einigen Algen und Pilzen,' chapter on Conjugatae, in which he shows (among other things) that they bear intense light very well, and that bright light is necessary for conjugation.

We have often noticed these cells begin to divide actively and ultimately produce new filaments (Figs. 62 and 65). That their activity is increased is also proved by the extraordinary development from these cells of swellings and processes, which so often occurs as an accompaniment to conjugation (cf. Fig. 58). Moreover, as previously mentioned, some cells by reason of this activity are induced to put out conjugating-tubes, which, not meeting with others, and not being able to fulfil their proper function, ultimately become rhizoids or organs of attachment.

Fam. I.—Zygnemaceae.

Sub-fam. 1.—*Mesocarpeae.*

This sub-family includes two genera, *Gonatonema*, comprising but four species, and *Mougeotia*, comprising upwards of thirty species.

1. **Mougeotia.** This genus now includes, and we think quite correctly, the genera *Mesocarpus*, *Craterospermum*, *Plagiospermum* and *Staurospermum*, all those characters regarded in the past as generic distinctions having been found by Wittrock[1] to be present in one species (*M. calcarea*). Many other observations also tend to prove the identity of these so-called genera. The conformation of the young zygospores of *M. uberosperma* (not taking into consideration the four outer processes) is decidedly that of a *Staurospermum*, whereas the adult zygospores are almost globose (cf. Figs. 42 and 43).

In this genus an axile plate-like chromatophore is present in each cell; and so far as our observations go, there is but one exception to this, *M. capucina* having an axile sub-irregular rod of chlorophyll connected to the lining primordial utricle by fine colourless threads of protoplasm. The rest of the cell-cavity between these meshes of protoplasm is filled with purple-coloured cell-sap[2]; the nucleus also stands out

[1] V. B. Wittrock, Om Gott. och Ol. Sotv. Alg., Bih. till K. Sv. Vet.-Akad. Handl., Bd. i, No. 1, Stockholm, 1872.

[2] Cf. remarks on this species by Lagerheim, Ueber das Phycoporphyrin, Vidensk.-Selsk. Skrift., I. Mathem.-natur. Kl., Kristiania, 1895, No. 5, p. 6 (Sep.).

very plainly, being opposed to the axile rod of chlorophyll towards the centre of the cell.

The method of conjugation and the formation of the rudimentary sporocarp are very well known, but we wish to point out a few irregularities which are occasionally met with. It is no uncommon thing for conjugation to take place through the end of one of the cells, the latter cell forming no conjugating-tube; we have observed this in *M. parvula* (Fig. 44) and *M. nummuloides*. We have also seen a hybrid example (Fig. 55), corresponding to *Spirogyra maxima*, var. *inaequalis* and others (Figs. 70 and 71), in which conjugation has taken place between two species of different thickness. Fig. 45 is an example of *M. recurva* in which three cells were conjugating to form one spore (analogous to other cases in *Spirogyra* and *Zygnema*; cf. Fig. 66).

There is a most noticeable disparity in size between the carpospores of different species in relation to the size of the sterile cells of the sporocarp (cf. Figs. 47 and 46 of *M. nummuloides* and *M. angolensis*, also similar remarks relating to the aplanospores of *Gonatonema*). An example of *M. capucina* from the New Forest is figured, in which there are two carpospores present in the same sporocarp (Fig. 48). This is analogous to the double zygospores of *Closterium lineatum* and certain abnormal cases of *Spirogyra* (Figs. 75 and 76). The carpospores of *M. irregularis* are worthy of note for the extreme irregularity of their spore-membrane (Figs. 56 and 57).

Spores resembling aplanospores are occasionally found in *Mougeotia*, but we have not been so fortunate as to meet with any. They are spores produced by the division of the original cell[1], and not by a rounding off of the contents as in *Gonatonema*; they may be regarded as carpospores formed from sporocarps (consisting of two or three cells) produced without conjugation, but possibly in consequence of the

[1] Wittrock, l. c., t. ii, f. 7 s, s (pseudospora tripartitione (more Staurospermi) sine copulatione formata), et 8 m, m (pseudospora bipartitione (more Mesocarpi) sine copulatione formata).

stimulus which has already caused conjugation to take place in a distant part of the filament.

Indications of sexuality are to be found in the Mesocarpeae, but they are much less marked than in the Zygnemeae. The spores are often seen to be nearer one filament, and the conjugating-tubes of that filament to be thicker and shorter than those of the other (cf. Fig. 47); hence the former may be looked upon as a female and the latter as a male filament. As these scarcely appreciable indications of sexuality are often absent, we may regard the Mesocarpeae as having lost almost all traces of differentiation into male and female gametes.

2. **Gonatonema.** The sterile specimens of this genus are undistinguishable from those of *Mougeotia*, although the chromatophore is more an axile rod (as in *Mougeotia capucina*) than an axile plate; the species of this genus are also of very much rarer occurrence than those of *Mougeotia*. The spores are asexual and parthenogenetic, and the whole contents of the cell are utilized in their formation.

During the formation of the spore and just before the appearance of the thin membrane round the cell-contents, we have noticed, both in *G. Boodlei* and *G. tropicum*, that in a few of the cells a more or less indistinct division of the cell-contents into two portions takes place. As to the precise import of this we cannot at present offer an opinion. Is it merely a chance arrangement of the cell-contents, or may it not be some slight retention of the last traces of ancestral sexual characters? Much is yet to be observed from the study of living *Gonatonema* during the active formation of spores.

It is also noticeable that the great difference in size between the spores of *G. Boodlei* and *G. tropicum* is more than can be accounted for by the difference in cubical capacity of the vegetative cells and contained cell-contents, the latter being almost the same in each case.

Figs. 1-15 illustrate the spore-formation in two species of *Gonatonema* which as yet have not been figured.

Sub-fam. 2.—*Pyxisporeae.*

This family is represented solely by the genus *Pyxispora* obtained from West Central Africa[1]. The vegetative cells, which are about 12–13·5 μ in thickness, contain two chromatophores very similar to those present in *Zygnema*, and in the sterile condition the plant could not be distinguished from the vegetative filaments of a species of the latter genus; each of these chromatophores has a small central pyrenoid. The conjugation is scalariform and similar to that present in the Mesocarpeae, resulting in an immediate tripartition into a sporocarp consisting of two sterile cells and an intervening carpospore.

The characters of this carpospore are unique, and sharply demarcate this genus from any other in the Zygnemaceae. It is broadly elliptical with rounded poles: it is disposed transversely to the longitudinal axes of the conjugating filaments, and around its edge, in the plane of its shorter diameter, is a small annular ridge marked by a circumscissile crack.

Some further figures of this interesting genus are given (Figs. 53 and 54).

Sub-fam. 3.—*Zygnemeae.*

This is the largest family of filamentous Conjugatae, and includes the five genera, *Zygnema*, *Pleurodiscus*, *Spirogyra*, *Sirogonium*, and *Debarya*.

The chromatophores of the genus *Spirogyra*, according to some botanical text-books, 'take the form of green spiral bands with toothed edges'; this is often true, but throughout the genus they exhibit much variation, there being every gradation between the slender, perfectly smooth spirals of *S. neglecta* with their axile uniform series of pyrenoids, and the broad serrated spirals of *S. nitida* and *S. porticalis*, containing scattered pyrenoids of various sizes. In fact, the

[1] West and G. S. West, Welw. Afric. Algae, Journ. Bot. 1897, p. 39.

characters of the chromatophores are not only remarkably constant but also widely different in many of the common species of the genus; those species with toothed edges to the chromatophores are however the most frequent.

The presence of straight chromatophores in the genus *Sirogonium* is in itself of no generic value, as those of *Spirogyra majuscula* are quite as straight, if not straighter, but the method of conjugation seems to us quite distinctive.

Owing to the somewhat irregular thickening of the walls of some species of *Zygnema*, such as *Z. ericetorum* and *Z. pachydermum*, and the more or less non-stellate condition of their chromatophores, they can be readily mistaken in the sterile condition for species of *Rhizoclonium* (a genus of Confervaceae Isogamae), and the short, few-celled branches of *Z. pachydermum*[1] render it still more liable to an error of this nature.

There are two modes of conjugation, *scalariform* and *lateral*, the details of which have been minutely followed out. In the former the cells of two or more filaments take part in the formation of the zygospores, but in the latter, conjugation takes place between the adjoining cells[2] of one filament only.

If conjugation is affecting only a portion of a filament, the increased activity along its whole length (as previously mentioned) often causes the cells of its free portions to develope conjugating-tubes, which, after making futile attempts to meet with a fellow, become more or less irregularly branched[3]; such is also the case in many examples in which conjugation has been interrupted.

On examining a large number of conjugated examples of *Spirogyra* or *Zygnema*, there is one prominent feature which at once strikes the observer, and on this point we cannot

[1] West, Algae from the West Indies, Journ. Linn. Soc. Bot. Vol. xxx, Pl. XIII, Figs. 12-15.

[2] In Cooke's Brit. Freshw. Alg., Pl. XXXI, f. 3 c, an example of lateral conjugation is shown between two non-adjacent cells.

[3] Cf. West, Sulla Coni. delle Zygn., Notarisia, 1891, Vol. vi, t. 12, Figs. 3, 5, 7, and 9.

do better than quote Bennett and Murray[1]. 'As De Bary has pointed out—and his statement is confirmed by nearly all more recent observers—the direction of conjugation is clearly governed by some physiological law, the movement of the protoplasm between the two filaments almost invariably taking place in one direction only, so that one of the two conjugating filaments is entirely emptied, while the other is filled with zygosperms.' In this paper we shall refer to the filament filled with zygosperms as the female, and the emptied one as the male filament.

As a rule a zygospore is formed by the fusion of the contents of two conjugating cells, but very rarely it is seen that three cells (two male and one female) have participated in its formation[2] (vide Fig. 66); in this way even three filaments may be concerned in the production of one zygospore. That this manner of conjugation is abnormal is proved by the larger number of failures than of completed attempts (vide Figs. 67 and 69). In those species belonging to the sub-genus *Zygogonium*, in which the zygospore is formed in the conjugating-tube, conjugation between three cells entails the production of two somewhat smaller zygospores, as in the example figured (Fig. 63).

Two filaments are generally concerned in an example of scalariform conjugation, but three, four, five, and even six are not uncommonly seen[3]. In such cases we have to deal with either polygamy or polyandry, and after the examination of hundreds of examples, we can confirm Bennett's statement that the former is rather more frequent, the ratio of the frequency of polygamy to polyandry being about 1·6 : 1.

During conjugation the filaments frequently assume a darker colour, this being most marked in *Spirogyra angolensis*, in which species they become blackish- or brownish-purple.

[1] Bennett and Murray, A Handbook of Cryptogamic Botany, p. 266.

[2] Cf. *Z. cruciatum* in West, Sulla Conj. delle Zygn., l. c., t. 13, f. 13; also *Spirogyra*, sp. in Borge, Siber. Chlorophy., Bih. t. Sv. Vet.-Akad. Handl., Bd. 17, Afd. 3, No. 2 (1891), t. i, f. 2.

[3] West, l. c., t. 12, f. 1.

Normal conjugation depends to a certain extent on the general surroundings of the filaments, many hindered and consequently irregular examples being met with in every gathering in an active state of conjugation. On rare occasions hybrids are produced, one species of *Spirogyra* conjugating with another of different thickness; examples of this are *S. maxima*, var. *inaequalis* [1], and some smaller species gathered in 1893 on Mitcham Common, Surrey (Figs. 70 and 71). Several abortive attempts at hybridism were seen in this gathering, the two examples figured being the only two observed with thick-walled zygospores. These spores were of variable form and dimensions, and were present in both filaments.

In contradiction to Cooke, we find scalariform conjugation to be much commoner than lateral conjugation, as may be gathered from the following table.

Species.	No. of gatherings of scalariform conjugation examined during the past few years.	No. of gatherings containing lateral conjugation examined in same period.
S. affinis	1	3
S. angolensis	1	
S. arcta	2	
S. bellis	6	1
S. calospora	1	
S. cataeniformis	4	
S. communis	5	
S. condensata	9	
S. crassa	9	1
S. cylindrospora	1	
S. decimina	3	
S. dubia	–	1
S. fusco-atra	1	
S. gracilis, and v. *flavescens*	13	1
S. Grevilleana	4	
S. inflata	1	2
S. insignis	4	
S. Jurgensii	2	1
S. longata	13	
S. Lutetiana	1	
S. majuscula (*S. orthospira*)	2	
S. maxima (*S. orbicularis*)	1	

[1] Wolle, Freshw. Alg. U. S., Pl. CXXXVIII, Figs. 5 and 6, and Pl. CXLII, Figs. 5 and 6.

Species.	No. of gatherings of scalariform conjugation examined during the past few years.	No. of gatherings containing lateral conjugation examined in same period.
S. neglecta v. *ternata*	1	1
S. nitida	17	
S. porticalis	3	
S. setiformis	2	
S. Spreciana	1	
S. tenuissima	–	16
S. varians	6	4
S. velata	1	
S. Weberi	7	1
S. Welwitschii	1	
Total	123	32

Lateral conjugation is much rarer in *Zygnema* than in *Spirogyra*, and although it is figured in various works, we have never yet seen an example of it. It is figured by Schmidle [1] in this genus in a species which he names *Zygnema* (*Zygogonium*) *Heydrichii*, but of which he gives no proper diagnosis [2].

Scalariform conjugation being far more predominant, we may say that lateral conjugation is the exception rather than the rule, and in view of this we may regard it with some amount of truth, as brought about by conditions unfavourable to conjugation in the natural or scalariform way. It may be thus considered to a certain extent as abnormal, and to what extent this abnormality is carried may be gathered from a consideration of Fig. 68 (drawn from an example from Mitcham Common, Surrey), in which specimen conjugation has taken place through the ends of the cells, and the conjugating cells have become genuflexed near their junction.

[1] W. Schmidle, Zur Entwickelung einer Zygnema und Calothrix, Flora, 1897, Bd. 84, Heft 2.

[2] This species seems to us to be only a *Zygnema spontaneum* with lateral conjugation. Nordstedt only found aplanospores when he described the species, but we have since found the zygospores (cf. Figs. 60 and 61) produced by scalariform conjugation (Journ. Bot., Feb. 1897, p. 40). The zygospores seen by Schmidle and produced by lateral conjugation agree in every way with those we saw in *Zygnema spontaneum*; moreover, the plants are of the same dimensions.

That it is not a perfectly normal condition is also proved by the numbers of failures where lateral conjugation was attempted [1].

Against the sexuality of the Zygnemeae only two plausible objections can be raised; these are the phenomena of *lateral* and *cross-conjugation*. It is clear that in the case of lateral conjugation sexual differentiation of the individual cells and not of the whole filaments must have taken place, and concerning this Bennett and Murray [2] state, 'that there is some differentiation of this kind would appear from the fact that when lateral conjugation takes place in a group of four cells the zygospores are formed in the two centre cells, which may be regarded as female.' This we find to be the case, as may be seen from the figures of *S. Jurgensii* and *S. inflata* (Figs. 72 and 73). Under certain conditions why should not this individual differentiation take place? Why should not the cells in a filament of *Spirogyra* or *Zygnema* be considered in a sense as only partially developed, further physiological changes taking place just antecedent to conjugation, which give the cells the characters either of a germ-cell or a sperm-cell? We see no reason for regarding the filaments as sexual until conjugation is about to commence, and then instead of the reproductive cells being specially cut off (as in *Temnogametum*), the contents of the individual cells undergo a profound physiological change, being imperceptibly converted into isogamous gametes. Also if the conditions of environment be such (as in an isolated filament) as to render it impossible for the whole of the cells of one filament to become of one sex, why should not individual sexuality of each cell be assumed? In some cases scalariform and lateral conjugation occur in the same filament [3], and within a few cells of each other. We have mentioned that a filament engaged in conjugation has the vigour of all its cells largely augmented, and may not the activity of the changes converting the ordinary vegetative

[1] West, Sulla Conj. delle Zygn., l. c., t. 12, f. 8.
[2] Bennett and Murray, Cryptogamic Botany, p. 267.
[3] Petit, Spirogyra des envir. de Paris (Paris, 1880), Plate I, f. 13.

cells into reproductive cells be so far modified at different parts of the same filaments that differentiation of sex is brought about? Regarding the cells in this light, each one may be considered as an individual plant; and why not? Each individual cell is capable of living apart from its neighbours, obtaining its own nourishment from the surrounding medium, and its life is in no way dependent upon the other cells of the filament. Moreover, if we allow that the Zygnemeae are comparable to the filamentous Desmidieae, we find that the latter readily dissociate into separate cells, which are not at all affected by their isolation (cf. p. 30 supra). The only important function of the assumption of a filamentous condition seems to us to be in the greater facility for conjugation afforded by the entanglement of the gregarious filaments.

Before considering cross-conjugation it will be as well to consider some examples of *interrupted conjugation*. A keen observer is continually coming across instances in which conjugation has by some means been brought to an abrupt termination before the proper formation of the zygospores has taken place, and in these cases the spores formed are very variable. It may be that something has caused a cessation of the activity along the whole filament, or that conjugation has been stopped between two cells only. Fig. 69 is an illustration of the latter, the forcible pressure of a second male conjugating-tube having narrowed the channel of communication to such an extent that union of the contents of the gametes was rendered impossible. The former is, however, much the most frequent. Sometimes the spore in the germ-cell is not of its true form [1], and occasionally two spores, one large and one small, are present in place of the normal one [2] (Figs. 75 and 76). When the conjugation has by some influence been hastened, a zygospore is often produced from only a portion

[1] Cf. *Spirogyra Groenlandica*, Rosenvinge in Öfvers. K. Vet.-Akad. Förh., 1883, No. 8, t. viii, f. 1–11.

[2] West, Sulla Conj. delle Zygn., l. c., T. XIII, Figs. 27, 28; binate spores in *Spirogyra communis*. A. Hansgirg, in Hedwigia, 1888, Hefte 9 u. 10, T. X, f. 6; binate spore in *Spirogyra Weberi*.

of the contents of the cells, and in these cases the spore is generally in the female filament (Fig. 74). Some examples have a spore in each conjugating cell (Figs. 77–80), and as a rule that in the female cell is of larger size. Occasionally the spores are of equal size (Fig. 78), and in rare cases the largest spore is in the male (?) cell.

All this leads up to cross-conjugation, which is the only other objection to sexuality. By *cross-conjugation* we mean scalariform conjugation with the formation of perfectly normal zygospores in each of the conjugating filaments, and we have seen but a solitary example of this amongst the thousands of conjugating specimens examined. This was a specimen of *Spirogyra gracilis* (Fig. 81) found in a gathering of Desmids obtained from a mass of *Utricularia minor* in a bog near Bowness, Westmoreland. As will be seen from the figure, there are two female cells and one male cell in one filament, and two male cells and one female in the other; moreover, the zygospores in each filament are perfectly normal, and the conjugation is complete and also normal. Now this is explicable, as in the case of lateral conjugation, by supposing that each individual cell has assumed sexuality. That the sexual condition of the filaments is the same in both lateral and cross-conjugation is proved by the occurrence of the former in both male and female filaments, which are also conjugating in a scalariform manner[1].

As a rule examples with zygospores in both filaments only exhibit a *false cross-conjugation* (Fig. 64), the zygospores in one filament being smaller than those in the other. This fact tends to prove that numerous attempts at cross-conjugation result in failures, normal zygospores not being produced, and together with its extreme rarity serves to show to what degree it is abnormal.

From the foregoing statements we have shown that lateral and cross-conjugation *are* explicable from a sexual point of view, and that there is no reason to regard the Zygnemeae as otherwise than sexual.

[1] Petit, in Bull. Soc. Botan. France, fevr. 1874, t. xxi, Pl. I, f. 2

Other minor observations have also been brought forward as a proof of this sexuality, such as the comparative lengths of the cells and the relative thickness of the male and female conjugating-tubes. These are, however, of little value, although it is certainly a fact that in the majority of instances the female conjugating-tube is shorter and thicker than the male (Figs. 66, 67, 79–81); also the female cells are often so swollen that all trace of a conjugating-tube is lost [1] (Figs. 67 and 81).

In a gathering of *Spirogyra velata* from a stream at Baildon, W. Yorks., several zygospores were noticed which did not assume a thick wall, but germinated immediately after their formation (Figs. 84 and 85).

The formation of spores without conjugation takes place not uncommonly in the Zygnemeae; these asexually-produced spores are called aplanospores, and are produced from the contents of a single cell. We have noticed them in *Zygnema leiospermum* (Fig. 83), *Z. pachydermum* [2] and *Spirogyra varians* (Fig. 82). They have been observed by Nordstedt [3] in *Zygnema spontaneum* and by Wille [4] in *Z. cruciatum*. In all cases the aplanospores are somewhat smaller than the zygospores, have a thinner membrane, and as a rule they are spherical, no matter what the form of the zygospore. We may also quote a remark made by Petit [5] concerning *Spirogyra mirabilis*, in which species the spores are produced without conjugation. He writes: 'Cette très curieuse espèce ne conjugue pas et ne laisse voir aucun tube copulateur; à une certaine époque de la vie de la plante, les cellules se renflent vers le milieu, l'endochrome se partage en deux parties qui se concentrent sous forme de globule aux deux extrémités de la cellule; il se forme ainsi une différentiation entre les parties de l'endo-

[1] Petit, Spirogyra des envir. de Paris, Pl. IX, f. 10; West, Freshw. Alg. W. Ireland, Journ. Linn. Soc. Bot., Vol. xxix, T. XVIII, f. 5.

[2] West, Alg. W. Indies, Journ. Linn. Soc. Bot., Vol. xxx, p. 266, Pl. XIII, f. 9, 10.

[3] O. Nordstedt, De Alg. aq. dulc. et Char. Sandvic, p. 17, T. I, f. 23, 24.

[4] N. Wille, Ferskv.-alg. Nov. Semlj. Öfvers. K. Vet.-Akad. Förh., 1897, No. 5, p. 63, T. XIV, f. 87.

[5] Petit, l. c. p. 14.

chrome. Bientôt les deux globules se rapprochent vers la partie renflée de la cellule et finissent par se réunir en constituant ainsi la zygospore.'

The vegetative cells of the genus *Debarya* are like those of *Mougeotia*. The conjugating-tubes of *D. glyptosperma* are long, some of them very long, and when they do not happen to meet with a fellow they often become club-shaped. As the cell-contents pass into the conjugating-tube the chromatophore takes the form of a loop, and a peculiar change comes over the empty cells as the zygospore is being formed; they become very clear and refractive, and a series of striations parallel to the transverse septa become visible. After this has taken place the cells have the appearance of solidity, this appearance being possibly due to annular thickenings deposited inside the cell-wall on the receding of the protoplasm during conjugation; in any case this character stands out distinctly in both old preserved specimens and in living ones. The large size of the zygospore is also a noticeable feature.

In *D. laevis* the conjugating-tubes are shorter and thicker and the spores are proportionately smaller. We give a figure of a specimen of this plant which has conjugated in a remarkable manner (Fig. 58). It has two to four pyrenoids in each chromatophore.

Mougeotiopsis, a genus recently described by Palla[1], seems to us to differ in no way from *Debarya*, except in the absence of pyrenoids. This is certainly in itself an insufficient generic character, and might probably be caused by the conditions under which the plants were growing. Lagerheim[2] states that *Mougeotia laevis* belongs to *Mougeotiopsis*; it is, however, a true species of *Debarya*, and as stated above certainly contains pyrenoids. The zygospores of both *Debarya laevis* and *Mougeotiopsis calospora* are scrobiculate, which seems to further indicate the identity of these two genera.

[1] E. Palla, Ueber eine neue, pyrenoidlose Art und Gattung der Conjugaten, Ber. der Deutsch. Botan. Gesellsch., Jahrg. xii (1894), Heft 8, pp. 228–236, T. XVIII.

[2] G. Lagerheim, Ueber das Phycoporphyrin, Vidensk.-Selsk. Skrift., I. mathem.-natur. Kl., Kristiania, 1895, No. 5, p. 16 (Sep.).

Fam. II.—TEMNOGAMETACEAE.

This order, defined as follows, 'Ordo novus *Conjugatarum*, conjugatio solum inter cellulas speciatim abstrictas,' was instituted a short time ago[1] to include a West African plant differing in a marked way from all the genera of Conjugatae. The sole representative plant is *Temnogametum heterosporum*, which has a great superficial resemblance to some species of *Mougeotia*. The vegetative cells are precisely like those of the latter genus, each cell being provided with a more or less plate-like chromatophore, in which a single series of from one to six small globose pyrenoids is embedded.

The conjugation is remarkable, owing to the fact that the reproductive cells are specially cut off from the rest of the plant; they are short, isogamous gametes, being about a quarter or a sixth part the length of the ordinary vegetative cells, and are cut off at intervals along the filaments. Some are cut off singly and others in pairs; in the former case the conjugation is scalariform, in the latter it is lateral. In scalariform conjugation the contiguous faces of the gametes become swollen, these swellings being merely short, rounded conjugating-tubes which finally unite (cf. Fig. 49), their union being followed by the bending towards each other and ultimate coalescence of the gametes to form a somewhat cruciate zygospore (Fig. 50). As previously mentioned[2], this zygospore at first sight very much resembles the central cell (or carpospore) of the five cells constituting the sporocarp of those species of *Mougeotia* belonging to the section Staurospermeae, but on closer examination the four contiguous cells are seen to possess their complete cell-contents, and to have taken no part in the formation of the zygospore. In the case of lateral conjugation, the pairs of cells become a little oblique or somewhat swollen on one side and then unite, this coalescence giving rise to an obliquely subcylindrical zygospore

[1] West and G. S. West, Welw. Afric. Algae, Journ. Bot., Feb. 1897, p. 37.
[2] West and G. S. West, l. c.

(Fig. 52), which has a considerable resemblance to the aplanospore of some species of *Gonatonema* (e. g. *G. notabile*). Soon after the coalescence of the gametes the wall of the zygospore increases much in thickness.

One case was noticed in which a solitary gamete in one filament was conjugating with one of a pair in another filament (Fig. 51).

There is no perceptible sexual differentiation between these gametes, but owing to the fact that they are specially cut off, this family must be regarded as considerably removed from the other families of the Conjugatae, though it is not so highly specialized as the Mesocarpeae.

Fam. III.—DESMIDIACEAE.

The chromatophores in the Desmidiaceae are disposed more or less symmetrically in the two halves of the cell, either as central masses of chlorophyll arranged in relation to certain pyrenoids, or as parietal and somewhat pulvinate masses containing scattered pyrenoids.

In some genera these pyrenoids are definite; the majority of Cosmaria have either one or two in each semi-cell, and the great majority of Staurastra contain one large one in the centre of each semi-cell. In a paper published by Lutkemuller[1] entitled 'Beobachtungen über die Chlorophyllkorper einiger Desmidiaceen' the author demonstrates the irregularity of the pyrenoids in certain species. No doubt many irregularities are to be found in most Desmids, but as a general rule we find the central pyrenoids very constant in character. One of the species mentioned by Lutkemuller as very variable in this respect is *Cosmarium pyramidatum*, normal specimens of which should contain two pyrenoids in each semi-cell. Our experience of this species confirms his observations, but we may add that in this respect it is the most variable species that we have yet examined.

[1] Oesterreich. Botan. Zeitschr., 43. Jahrg. 1893, No. 1.

Specimens of *Cosmarium ornatum* occasionally have irregular pyrenoids, but we have not yet seen more than one example in a hundred. Amongst an immense number of examples of *Cosmarium sphagnicolum*, collected in early spring in N. Yorks. from moorland pools nearly filled with *Sphagnum cuspidatum* and *Ptilidium ciliare*, many variations were observed in the chromatophores, and the pyrenoids which were normally one in each semi-cell, varied from one to three (cf. Figs. 34–36). This variability of the chromatophores has been described in *Penium minutum*[1].

We figure four cells of *Hyalotheca neglecta* (Figs. 30–33), which show considerable variation in the pyrenoids, but these were only found after the examination of a very large number of filaments. This irregularity is usually found after rapid division, in the same way that abnormality of form is probably caused (cf. Fig. 39 of *Euastrum didelta*).

This subject is one concerning which much work is yet desirable, as several doubtful genera have been founded on the structure and arrangement of the chromatophores; e. g. *Pleurotaeniopsis*, *Pleurenterium*, &c.

Sexual (?) reproduction is by conjugation and formation of zygospores, the conjugating cells generally not being differentiated. Double spores are formed in *Closterium lineatum*, *Cylindrocystis diplospora* and *Penium didymocarpum*, and are analogous to those in the Mesocarpeae and homologous with those in the Zygnemeae. In some rare cases three (or even four) cells have participated in the formation of a zygospore[2]. The zygospore is formed *between* the conjugating cells in all Desmids except *Desmidium cylindricum*. In this species it is formed *within* the female cell[3] as in *Spirogyra*

[1] Cf. remarks by Lutkemuller under *Docidium baculum*. Also Journ. Bot., March, 1895, p. 65.

[2] Cf. *Staurastrum teliferum* in West, Freshw. Alg. W. Ireland, Journ. Linn. Soc. Bot., Vol. xxix, Pl. XXIV, f. 5; *Cosmarium rectisporum*, W. B. Turner, Freshw. Alg. E. India, K. Sv. Vet.-Akad. Handl., Bd. xxv, No. 5, T. X, f. 16e; also *Closterium Pritchardianum*, West and G. S. West, Freshw. Alg. S. of England, Journ. Royal Micr. Soc., Pl. VI, Fig. 5, December, 1897.

[3] Ralfs, Brit. Desm., T. II, f. 1, e, f, g, h, i, k; Wolle, Desm. U. S., Pl. III, f. 4;

and *Zygnema*, although, as in most filamentous Desmidieae, the filaments break up before conjugation. So far as we know, this is the only case of differentiation of the conjugating cells met with in the whole of the Desmidiaceae. Boldt[1] figures a 'forma monstrosa' of *Hyalotheca dissiliens* with the zygospore in one of the cells, and Joshua[2] mentions a case where *Hyalotheca dissiliens* was conjugated like *Desmidium cylindricum*. Fig. 37 is also an approximation to this stage in an example of *Hyalotheca dissiliens*. What is this 'monstrous form' of conjugation in this species? Abnormal it certainly is as compared with ordinary conjugated examples, but is it not a case of reversion to some ancestral type of conjugation, represented at present by the Zygnemeae, and which the Desmidieae have almost lost, the lingering remains of which are still found in *Desmidium cylindricum*?

Thus degeneration and loss of sexual differentiation of the conjugating cells have gone on hand in hand with the loss of the filamentous condition, the majority of filamentous forms dissociating before conjugation. An extreme morphological specialization has accompanied this loss of the filamentous condition, causing the large majority of this family of unicellular plants to be remarkable for their beauty and variety of form.

In the genus *Desmidium* conjugating-tubes are formed, and we have noticed rudimentary conjugating-tubes in some species of *Closterium*[3] and in *Arthrodesmus octocornis*.

Conjugation seems to take place in many Desmids immediately after division and before the young semi-cells have had time to attain maturity[4]: for sexuality to exist

West and G. S. West, N. Amer. Desm., Trans Linn. Soc. Bot., ser. 2, Vol. v, Pt v, Pl XII, f 29

[1] K. Boldt, Desm fran Grønl., Bih. till Sv. Vet.-Akad. Handl., Bd. xiii, Afd. 3, No 5, T II, f. 33

[2] W Joshua, Notes on Brit Desm., Journ. Bot., Vol. xx 1882.

[3] In *Closterium Ehrenbergii* they are perforated protuberances at the base of the younger semi-cells; cf. West and G. S. West, Journ. Roy. Micr. Soc., 1896, p. 151.

[4] West and G. S. West, l. c., pp. 151 and 153, Pl. III, f. 29; also W. Archer, in Quart. Journ Micr. Soc., Vol ii, p. 251.

under these conditions, the physiological change (previously referred to) from the vegetative to the reproductive cell must be immediately antecedent to conjugation.

Lateral conjugation is not unknown amongst filamentous Desmids. Ralfs describes[1] the conjugation of two adjacent cells in a filament of *Sphaerozosma excavatum* as taking place between their flat ends, and we have seen an example of this in *Spondylosium pulchrum*, var. *planum*, from Orono, Maine, U.S.A. In these instances the filament does not fragment before conjugation, the zygospore filling up the space originally occupied by the two adjacent semi-cells of the conjugating cells.

Aplanospores are occasionally found in the Desmidieae; Bennett[2] mentions the occurrence of some spore-like bodies produced without conjugation in *Closterium*, and they are figured by Wallich[3] and Turner[4] in *Spondylosium nitens*. In a gathering of Desmids from the New Forest in which *Hyalotheca neglecta* was abundant, many of the cells contained aplanospores (Cf. Figs. 23–27); these were produced by the rounding off of the cell-contents and final assumption of a thick cell-wall. They differ in form from the globose zygospores, being elliptical, with rounded poles, and when mature their walls turn yellowish-brown.

Phylogeny.

In all probability the Zygnemaceae have arisen along two distinct lines from some ancestral filamentous sexual Conjugates. The Mesocarpeae may have been developed through *Debarya* along one of these lines, and from them *Temnogametum* probably struck off at some early stage. Along the other line the remainder of the existing Conjugates

[1] Ralfs, Brit. Desm., p. 67.

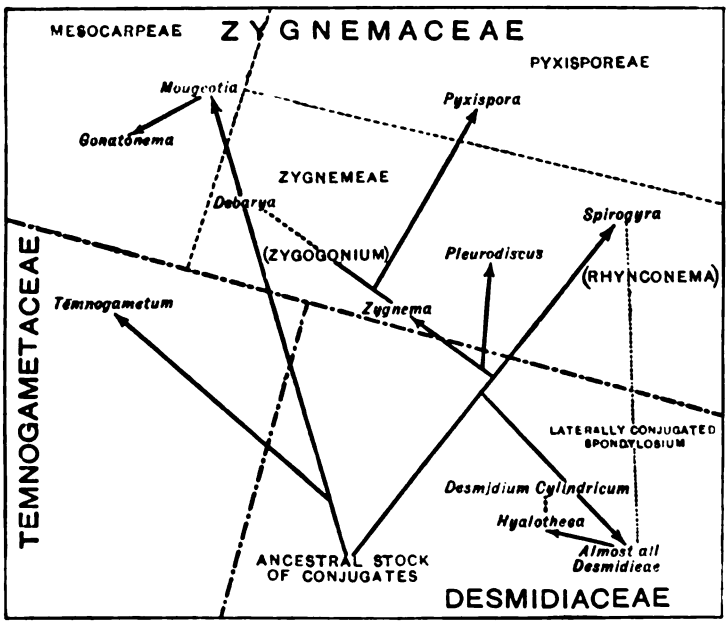
[2] A. W. Bennett, in Annals of Botany, Vol. vi, No. 21, April, 1892.

[3] G. C. Wallich, Desm. Low. Bengal, Ann. Mag. Nat. Hist., Ser. iii, Vol. v, 1860, T. VII, f. 10, 11.

[4] W. B. Turner, Freshw. Alg. E. India, K. Sv. Vet.-Akad. Handl., Bd. xxv, No. 5, T. XVIII, f. 7, 8.

were probably developed, and at a short period before the Zygnemeae became differentiated into the two distinct groups represented by *Spirogyra* and *Zygnema*, the Desmidieae were probably evolved by retrogression. The view that the latter may have been evolved in this way is confirmed by the occasional reversion of *Hyalotheca* to its ancestral mode of conjugation, the remains of which are still found in *Desmidium*

TABLE OF PHYLOGENY.



cylindricum. *Pleurodiscus*, which conjugates like a *Zygnema*, was probably evolved from the *Zygnema* group just after its differentiation from the *Spirogyra* group, *Pyxispora* being evolved from *Zygnema* by the assumption of a rudimentary sporophyte-generation, thus placing it on an equal level of specialization with the Mesocarpeae. *Zygnema* can be connected with *Debarya* by the subgenus *Zygogonium*, and

a parallelism of modification has gone on in the reproduction of *Spirogyra* and certain filamentous Desmids, as shown by the obsolete *Rhynchonema* and the laterally conjugated *Spondylosium*.

We cannot but regard the Mesocarpeae and the Pyxisporeae as the most highly specialized families of the Conjugatae, the formation of the sporocarp being a faint indication of an 'alternation of generations[1].'

There is a little retrogression in the Mesocarpeae, certain plants of this family (placed under a distinct genus—*Gonatonema*) producing spores only asexually. The accompanying phylogenetic table has been drawn up to graphically illustrate the conclusions we have arrived at.

DESCRIPTION OF PLATES IV AND V.

Illustrating Messrs. West's paper on Conjugatae.

PLATE IV.

Figs. 1–9. *Gonatonema Boodlei*, West and G. S. West. From Mitcham Common, Surrey. × 520.

Figs. 10–15. *Gonatonema tropicum*, West and G. S. West. From Huilla, Angola, W. Africa. × 520.

Fig. 16. *Mougeotia sp.* Showing organ of attachment. × 120.

Figs. 17–19. *Mougeotia sp.* Showing short lateral branches. × 120. From Frizinghall, W. Yorks.

Figs. 20, 21. *Spirogyra sp.* With rhizoids; from Hanka Deela, Somaliland. × 120.

Figs. 22–33. *Hyalotheca neglecta*, Racib. × 520. From the New Forest, Hants. 22, vegetative filament with wide gelatinous sheath; 23–27, showing formation of aplanospores; 28–29, zygospores; 30–33, single cells with irregularity of pyrenoids.

Figs. 34–36. *Cosmarium sphagnicolum*, West and G. S. West. × 520. From Mossdale Moor, Widdale Fell, N. Yorks. 34, with normal pyrenoids; 35 and 36, with irregular pyrenoids.

[1] The Mesocarpeae afford a better example amongst the lower Algae of a sporophyte-generation and a rudimentary alternation of generations than that shown by the Oedogoniaceae.

Fig. 37. *Hyalotheca dissiliens* (Sm.), Breb. A peculiarly conjugated example from Thursley Common, Surrey. × 250.

Fig. 38. *Euastrum binale* (Turp.), Ehrenb. Showing tendency to assume a filamentous condition; from Thursley Common, Surrey. × 250.

Fig. 39. *Euastrum didelta* (Turp.), Ralfs. Specimen from Wrynose, Lake District, abnormally divided. × 220.

Fig. 40. *Tetmemorus granulatus* (Breb.), Ralfs. Specimen from the New Forest, Hants, one semi-cell branched. × 250.

Fig. 41. *Mougeotia sp.* Showing branching; from near Lindley Reservoir, W. Yorks. × 250.

Fig. 42. *Mougeotia uberosperma*, West and G. S. West. With immature spores. × 520.

Fig. 43. *Mougeotia uberosperma*, West and G. S. West. With mature spores. × 520.

Fig. 44. *Mougeotia parvula*, Hass. From Black Hill, near Settle, W. Yorks. × 520.

Fig. 45. *Mougeotia recurva* (Hass.), De Toni. Three cells conjugating together; from Borrowdale, Lake District. × 520.

Fig. 46. *Mougeotia angolensis*, West and G. S. West. From Pungo Andongo, Angola, W. Africa. × 250.

Fig. 47. *Mougeotia nummuloides*, Hass. From Scarf Gap Pass, Lake District. × 250.

Fig. 48. *Mougeotia capucina* (Bory.), Ag. Example from New Forest, Hants, with two carpospores. × 250.

PLATE V.

Figs. 49–52. *Temnogametum heterosporum*, West and G. S. West. From Huilla, Angola, W. Africa. × 250.

Figs. 53, 54. *Pyxispora mirabilis*, West and G. S. West. From Huilla, Angola, W. Africa. × 520.

Fig. 55. A hybrid specimen from Strensall Common, N. Yorks.; conjugation taking place between two species of *Mougeotia*. × 400.

Figs. 56, 57. *Mougeotia irregularis*, West and G. S. West. From Pungo Andongo, Angola, W. Africa. × 350.

Fig. 58. *Debarya laevis* (Kutz.), West and G. S. West. Peculiarly conjugated example from Mitcham Common, Surrey. × 220.

Fig. 59. *Debarya laevis*. Mature zygospore showing the scrobiculations. × 520.

Figs. 60, 61. *Zygnema spontaneum*, Nordst. Mature zygospores produced by scalariform conjugation; specimens from Huilla, Angola, W. Africa. × 520.

Figs. 62, 63. *Zygnema pectinatum* (Vauch.), Kutz. 62, × 120; 63, × 220.

Fig. 64. *Spirogyra condensata* (Vauch.), Kutz. × 120.

Fig. 65. *Zygnema pectinatum* (Vauch.), Kutz. × 120.

Fig. 66. *Spirogyra maxima* (Hass.), Wittr. × 100. Conjugation between three cells.

Fig. 67. *Spirogyra sp.* From Huilla, Angola, W. Africa. × 120.

Fig. 68. *Spirogyra inflata* (Vauch.), Rabenh. × 220. From Mitcham Common, Surrey.

Fig. 69. *Spirogyra condensata* (Vauch.), Kutz. × 250.

Figs. 70, 71. Hybrids from Mitcham Common, Surrey. × 250. Conjugation between two species of *Spirogyra* of different thickness.

Fig. 72. *Spirogyra inflata* (Vauch.), Rabenh. × 100.

Fig. 73. *Spirogyra Jurgensii*, Rabenh. × 100.

Figs. 74, 75. *Spirogyra bellis* (Hass.), Crouan. × 140.

Fig. 76. *Spirogyra velata*, Nordst. × 220.

Figs. 77, 78. *Spirogyra bellis* (Hass.), Crouan. × 140.

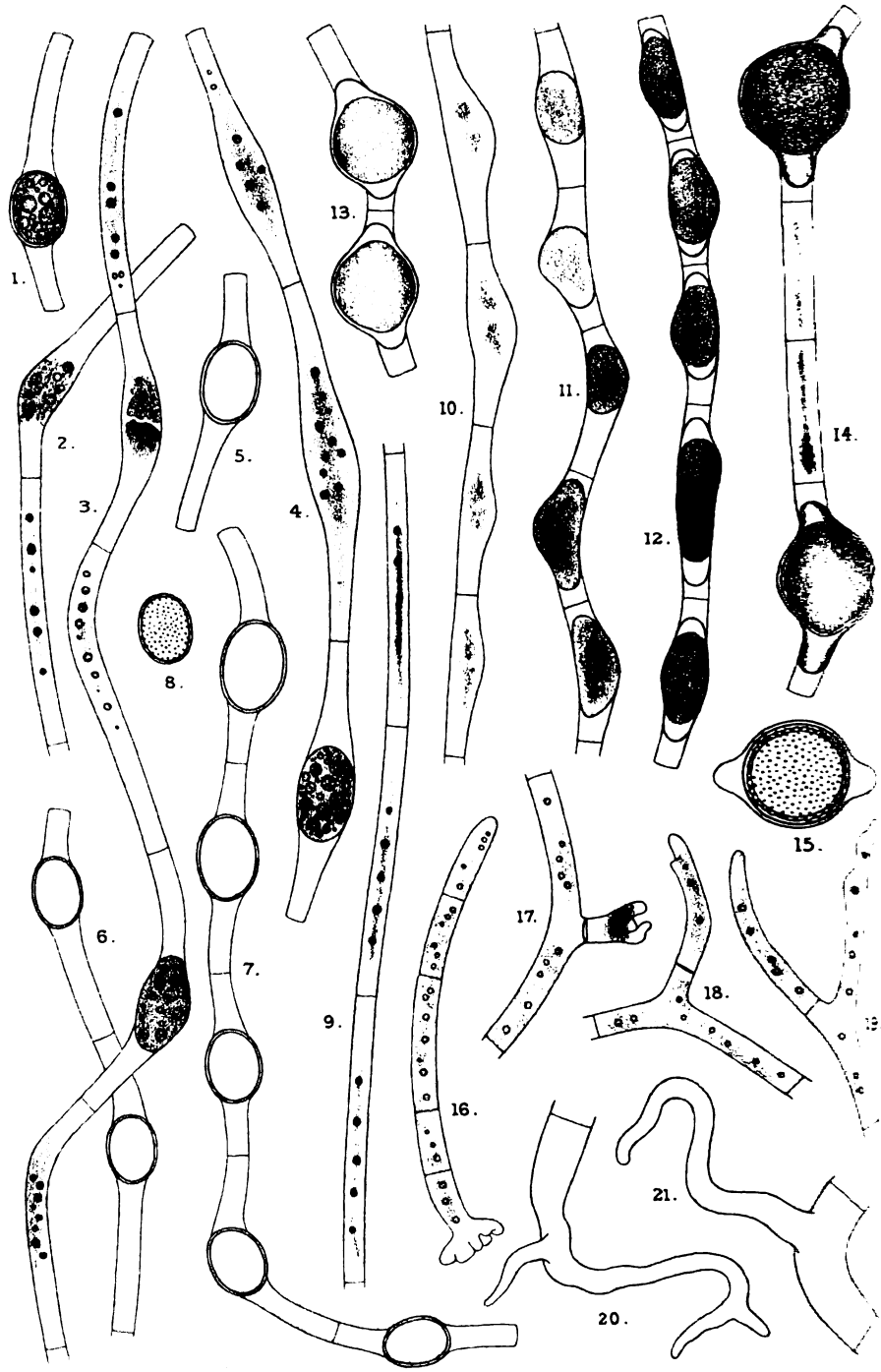
Figs. 79, 80. *Spirogyra velata*, Nordst. × 220.

Fig. 81. *Spirogyra gracilis* (Hass.), Kutz. forma. × 350.

Fig. 82. *Spirogyra varians* (Hass.), Kutz. × 520. Aplanospore.

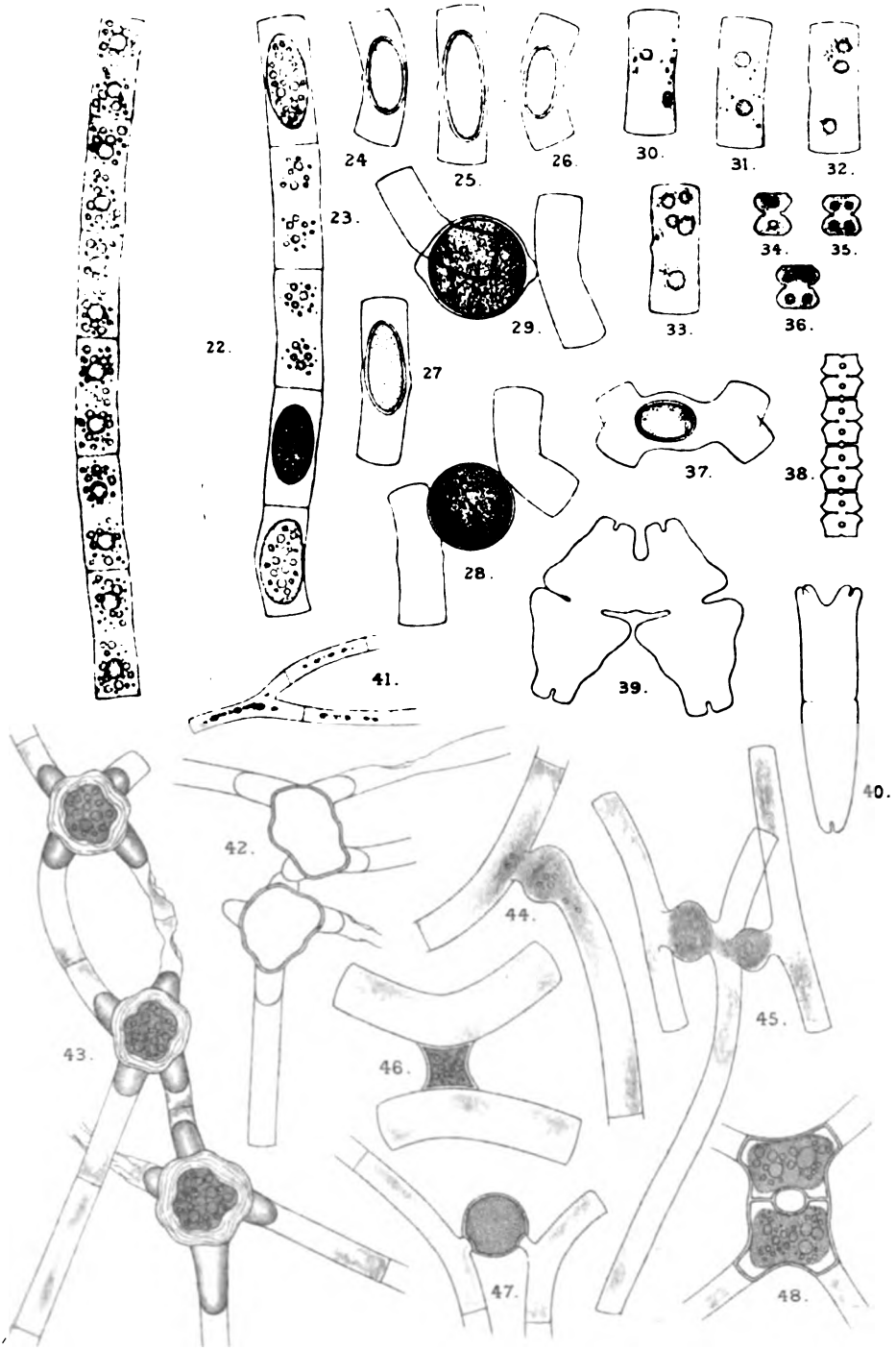
Fig. 83. *Zygnema leiospermum*, De Bary. × 520. Showing aplanospore.

Figs. 84, 85. *Spirogyra velata*, Nordst. Two zygospores germinating immediately after formation. × 220.

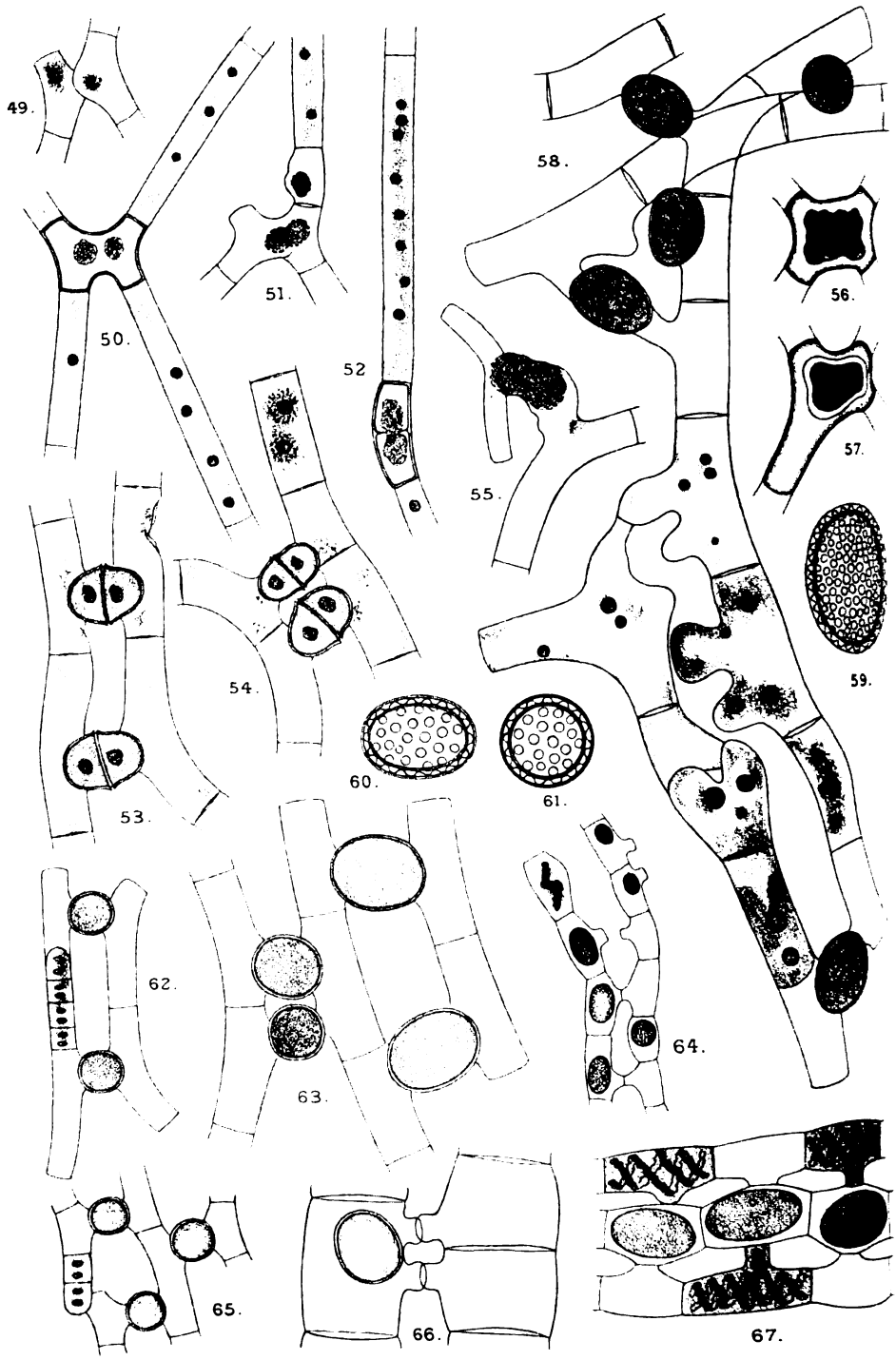


G.S.West ad nat.del.

WEST. — CONJUGATAE.

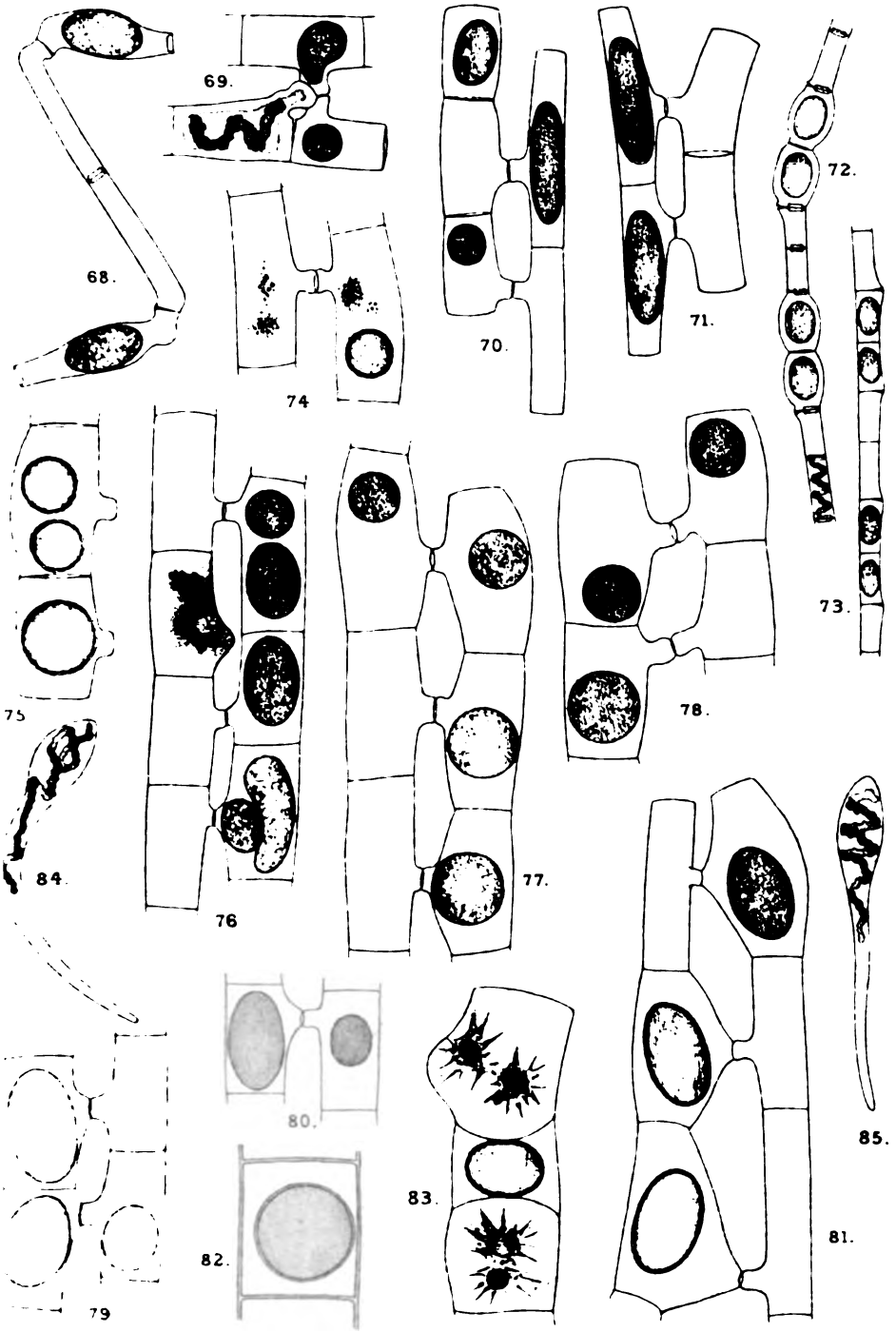


University Press, Oxford



G. S. West. ad. nat. del.

WEST. — CONJUGATAE.



University Press, Oxford

A Violet Bacillus from the Thames.

BY

H. MARSHALL WARD, Sc.D., F.R.S.

Professor of Botany in the University of Cambridge.

With Plate VI.

ONE of the most interesting forms of Schizomycete I have isolated from the Thames[1] is a bacillar or filamentous one observed more especially in the winter, though probably present also in the summer with altered characters.

It is remarkable for producing in vigorous cultures a magnificent violet pigment of a peculiarly pure hue; in fact almost exactly the colour of dark blue violets, or of a strong solution of gentian-violet.

This species grows well and is easily cultivated at all temperatures from about 10° C. to 25° C., on all the ordinary solid media, and in milk and broth, though difficulties are occasionally met with in potato-cultures. Specimens in gelatine and broth-drops grow out as long filaments, often 50 to 60 μ and longer, which move with an oscillating and sinuous motion, or progress slowly across the field with similar movements. These filaments are obviously segmented,

[1] See Proc. Roy. Soc., Vol. lxi, 1897, p. 415. The form here described is that constituting the type of Group II on p. 417.

[Annals of Botany, Vol. XII. No. XLV. March, 1898.]

and one can see them break at the septa into bacillar rodlets from 3 to 5 or even 7 μ long by about 0·75 to 0·8 μ broad or a little more (Fig. 3). They are perfectly colourless, and move more rapidly than the filaments, often progressing straight forward with fairly rapid motions right across the field—say a distance of 100 to 200 times their own length,—and then suddenly stop and dart backwards a short distance, and then on again. Sometimes these progressive movements are associated with a spinning on the axis, or about a central point, but more often they are straight forwards.

Specimens stained with methyl-violet show similar sizes, but if taken from older gelatine-cultures the rodlets may be very short—about 1 μ long by 0·75 to 0·8 μ broad—and almost like cocci (Fig. 1). After a year in culture, the rods are often thinner, 0·6 μ or so (Fig. 2), but soon thicken up in the broth-drop at ordinary temperatures. No trace of spores could be detected; but from some experiments where old cultures were maintained at 50° C. to 60° C. for several hours, it appears not improbable that the coccus-like joints act in the capacity of spores, and can withstand fairly high temperatures for a short time. If this turns out to be correct, this Schizomycete may have to be classed as a *Bacterium* and not as a *Bacillus*. The rods often show three or four spore-like, bright, denser spots (Figs. 1, 2).

Numerous attempts to obtain any further development in culture-cells led to negative results only. In gelatine-drops at 18–22° C. the growth to filaments occurs as in broth, but more slowly, and in twenty-four hours the rods and filaments are motile, slowly writhing or jerking in the liquefying drop; the movements are obviously restricted.

Plate-cultures in gelatine at 20° C. show colonies in forty-eight hours—visible to the unaided eye on the third day—which are small, slightly yellowish discs, with a granular texture under a one-third objective and sometimes traces of zonal structure where submerged. Those nearer the surface break through in a conical shape, and spread their margins irregularly over the gelatine: the much more extended

surfaces of these colonies appear granulated and rugose, as if marked with a delicate and complicated series of contours and mounds, giving a characteristic aspect, which, however, is by no means peculiar to this specific form—e. g. such colonies occur in Typhoid (Fig. 5).

In four or five days the colonies appear as milk-white, opaque, thin, discoid, smooth and glistening expansions to the unaided eye, though the microscope shows the same characters as described above. They grow very slowly, and even after ten days at 20° C. may be still white, though somewhat thicker, like an opaque, flattened milky drop, about 4 to 5 mm. in diameter, and slightly sinking in the now softening gelatine. They are, in fact, beginning to liquefy the latter.

About this period, also, the superficial colonies are usually beginning to show the violet pigment—though sometimes it occurs much earlier and at others is delayed—the colour appearing first as a mere trace of purplish cast on a dirty-white matrix, gradually spreading over the colony, till the latter is deep violet or almost blue black. The pigment does not spread into the gelatine, but is strictly confined to these superficial colonies, at least for a long time. The totally submerged colonies may remain dirty-yellow, or buff-coloured, even for weeks, so that oxygen may have to do with the development of the pigment; in some cases, however, I have found them faintly purple in four or five days (cf. Figs. 4–6).

It is interesting to note that slow growth, with the formation of the pigment, occurs at temperatures even as low as 5° or 6° C. on gelatine-plates kept cool by ice.

If there are very few colonies on the plate, they may eventually dry up with the non-liquefied gelatine, and I have plates in the laboratory more than two years old where this has occurred. The hard, smooth, deep purple discoid colonies on these plates look just like dried ink-drops, each about 10–12 mm. in diameter, with irregular margins, and each surrounded with a pale zone, 2–6 mm. broad, scooped out of the yellower dry gelatine, and representing where liquefaction

occurred. The water having evaporated, the colonies are flattened on to the glass.

In stab-cultures in gelatine a white, button-like mass forms at the infection-point in three days at 15° C., while minute points develop along the line of inoculation. In ten days a thistle-head funnel of liquefaction is developed, still all quite white. In eighteen days the liquefaction at the top has reached the walls of the tube, or nearly so, and the liquefied gelatine above is found to have a dense, tough membrane on its surface, the white matrix of which is turning purple. This membrane (zoogloea) lines the sides of the funnel-shaped depression, as the surface of the gelatine sinks owing to evaporation (Fig. 7 *c*).

In a month, at this temperature, the gelatine was liquefied about one-eighth of the distance down, and a deep violet zoogloea-membrane floated on the top, while violet flecks had fallen to the bottom of the liquid, and rested on the still solid gelatine below (Fig. 7 *d*).

All the gelatine-tube cultures show liquefaction of the gelatine. The rate at which this progresses at 20° C. may be estimated from the following: in six days the upper one-third of the gelatine of a stab-culture was liquefied, and with a beautiful deep violet, funnel-shaped mass of the Schizomycete tailing off below into the solid gelatine. Even after three months the gelatine is only liquefied about two-thirds down the tube, and six months' tubes may still have some gelatine solid below. The liquefied gelatine is very viscid. The thin streak in the solid gelatine was still buff in colour, apparently from lack of oxygen.

Streak-cultures on gelatine, at 15°–20° C., grow fairly rapidly. In twenty-four hours at 15° C. a white opaque streak was developed, which in three days began to show traces of the violet pigment, and the gelatine began to soften below, so that the culture sank in. In nine days the gelatine was excavated by a large scoop-like cavity, filled with viscid liquid (the surface being kept flat by inclining the tube till nearly horizontal) on which floated a deep violet membrane, while

in the liquid itself flecks of white and violet colonies were floating or sunk to the bottom (Fig. 8). In three or four months all the gelatine is liquefied, and dirty-violet flecks are floating in the liquid

Closer examination shows that the violet mass is composed of a complex folded and wrinkled zoogloea-form, and that the pigment is confined entirely to this, and does not diffuse into the liquefied gelatine in which the trembling jelly-like mass floats. These zoogloea-masses are found scooping out cavities in the otherwise solid gelatine, and lining them with the folded membrane. Later on, the liquefaction slowly spreads away from the zoogloea, but the pigment is confined to the latter. The same occurs, somewhat more rapidly, at 20° C. On removing the zoogloea-membrane, these cavities are left apparently devoid of Bacilli; but the microscope shows their presence in abundance, and in a few days those remaining have multiplied and again covered the concave surfaces with the membrane, which soon thickens, wrinkles, and obtains the violet colour as before. When the gelatine is completely liquefied, the violet colour is confined to the membrane at the surface, or to pieces of it which sink in: the non-oxygenated submerged Bacilli forming a precipitate at the bottom are buff or greyish-yellow, or even dirty-white.

On agar, at 20° C., a thick white or slightly buff opaque streak forms in twenty-four hours or so, and in three days has a waxy appearance, often with raised margins. On the fourth day the purple colour begins to show here and there in the white, and in ten days the whole surface of the agar may be covered with an intensely deep purple, corrugated membrane, which is very tough and may be lifted off bodily from the medium. Underneath, the growth is still white, and the colour is confined to the zoogloea-mass—it never diffuses into the agar (Fig. 9). In feeble cultures the growth may be so slow as scarcely to extend beyond the path traced by the inoculating needle; but here too, the deep, ink-like, purple-black drops soon appear as before.

On potatoes, at 20 C., a thickish, dirty-white streak, tinged

with grey or yellowish-brown, forms in from two to five days, and slowly spreads; it varies considerably as to thickness, wet or dry appearance, and rate of growth (Fig. 10). Generally, but not always, the violet colour begins to appear about the fourth day, and then invades the mass, and a tough, deep violet, corrugated and mamillated membrane is formed as on agar, but not so extensively. In some cases the cultures on potatoes fail altogether to produce the pigment, and I am unable to say why. Very often the violet colour does not extend to the edges, but remains towards the centre.

In broth, at 20° C., turbidity is just perceptible on the second day, and this increases. Later on a thick membrane forms above, the liquid below being very turbid. The membrane is at first white, and may remain so for ten days or more. In strong cultures the violet hue appears sooner or later, and in twenty days there is a tough, much corrugated, violet membrane floating on the still turbid liquid, at the bottom of which a white deposit collects. The colour of the membrane deepens as the culture ages, and it clings so tightly to the walls of the tube that the liquid does not escape on upturning.

Broth at 35° gives no growth in four or five days—the liquid is perfectly clear.

Carrot at 25° C. Spreads as a white film in forty-eight hours, and in three days is a purple and white paste. In fifteen days much spread as a white, wet, pasty layer, but only small patches of purple here and there.

Artichoke at 25° C. Much as on carrot, but perhaps more waxy. In fifteen days the white wet paste is smeared with blue-violet patches.

Milk undergoes slight coagulation, and the separated casein is slowly peptonized, or peptonization may occur without evident precipitation. The reaction of the liquid is alkaline. After some time—it may be three or four weeks—the violet pellicle forms on the surface, deepening in colour from day to day. In some cases, at 25° C., traces of violet occur above in a week, and in the deposit in a fortnight, and still no change is evident in the milk, except a grey colouration and

marked alkalinity. The grey colour is due to the spreading of the purple through the milk. In a month nearly all is peptonized, and the liquid purple and very strongly alkaline.

The colouring pigment cannot be detected in the Bacilli themselves under the microscope, but seems to be in the membrane—the swollen cell-walls forming the zoogloea-matrix —or even external to this. It is hardly, if at all, soluble in water, as is shown by the fact that on filtering water in which large quantities of the purple flecks &c. have been crushed, the liquid comes off quite or nearly clear; but it is readily dissolved out by absolute alcohol. This alcoholic solution is, moreover, extremely stable, and I have kept a tube half full of it for more than six months unaltered in the dark at ordinary (spring and summer) temperatures. The beautiful blue-violet colour has a slight reddish cast in it, reminding one exactly of a solution of gentian-violet.

The alcoholic solution shows one broad absorption-band, extending from the red to the green-blue; but even a layer half-an-inch thick lets some of all the rays through.

Acetic acid slowly renders the alcoholic solution paler. NaHO turns it bluish-green, the violet colour returning, but paler, with slight excess of HCl.[1] On evaporating the alcoholic solution to dryness over a water-bath, the purple sediment dissolves up again in alcohol apparently unaltered. Stable as it thus is, however, the solution exposed to the bright sunlight of an August day is completely bleached in from one to two hours.

Old milk-cultures yielded Bacilli, the gelatine-colonies from which grew well, but were quite colourless, and very like those of *B. Coli communis*. It required several passages through broth and gelatine to get the colour up again.

I have made numerous experiments with this Schizomycete to determine its relations to light, and find it one of the most sensitive as yet tried. It is quite easy to obtain sun-prints with agar-plates over which a stencil-letter is placed, with

[1] Practically the same reactions were observed by Macé with the violet pigment from his *B. violaceus* (*Traité pratique de Bactériologie*, 1892, p. 541).

a couple of hours' exposure on a spring day. I have also attained fairly satisfactory results in bright sunshine by placing a plate behind an ordinary photographic negative: the print was slightly wanting in sharpness, because of course I had to have a plate of sterilized glass between the agar-film and the negative; but in cases where the glass was thin enough the picture was very good indeed.

Tubes of water containing large quantities of this Bacillus, can be almost completely sterilized by a few hours' exposure to sunshine. No doubt the question of temperature comes in in all these cases, but it must be noted (1) that I have kept them alive for four or five hours in a hanging-drop at 30 to 35° C., and (2) that in the experiments with the solar and electric spectra, where the exposures are made over ice, the maximum light-effect is not towards the red end at all, but in the blue-violet. I therefore regard these bactericidal effects as due to the blue-violet light-rays, and not to the high temperature.

I have made numerous attempts to watch and measure the growth and division of the rodlets, but although I have been able to convince myself that the filaments segment up into the short rodlets, the fact that both filaments and rodlets are moving actively in all the available media, as soon as the light is turned on them[1], though in some cases at any rate they seem to become quiescent again in the dark, has completely baffled all my endeavours. These movements seem to depend on a fairly high temperature—20 to 25° C. or so—and I should say the divisions are completed about every twenty minutes or so[2]. The shorter segments (Bacilli) often move actively, each for itself, before separating from the other segments—rarely more than four in all—of the filament, which has a slow, undulating movement as a whole. I have not investigated the question of cilia, but the character of the movements of

[1] This fact had already been observed by Engelmann (Unters. aus dem Physiol. Lab. zu Utrecht, 1882, p. 252).

[2] Brefeld (Bot. Unters. IV, p. 46) found that *B. subtilis*, at 24° R. and under favourable conditions, divided once every thirty minutes.

the individual Bacilli is such as to lead one to expect that each has one or more.

As already stated, I have entirely failed, after many attempts, to make this Schizomycete develop recognizable spores. In old cultures on agar or gelatine the rods and filaments, both long and short, are usually found to contain small stainable bodies, which, owing to their brightness and general appearance in the fresh preparations, are often very like spores; especially when, as frequently occurs, they lie singly in the rodlets or at regular intervals in the filaments. These are not spores, however, for they stain as readily with the ordinary bacillar dyes—such as methylene-blue and methyl-violet—as they do with carbolized fuchsin, and aniline, methyl-violet, &c. Moreover, these stainable masses are of all sizes, and in all positions; there is nothing definite or spore-like about them. In old cultures they are also often met with in the interior of giant rods and filaments of irregular shapes, which are obviously of the nature of the so-called involution forms.

All attempts to get them to germinate have failed, and they do not withstand high temperatures: moreover, the rodlets and filaments containing them are still capable of further growth and division when placed in hanging-drops. On the evidence, therefore, I conclude that no spores are developed under ordinary conditions.

The following table exhibits a summary of the characters.

VIOLET BACILLUS.

Habitat.	*In the Thames, especially in winter, and not a common form.*
Morphological characters.	Rodlets or filaments, from 2–3 μ to 60 μ and upwards long by about 0·75 to 0·8 μ broad. Often quiescent, but may be actively motile. Involution-forms in old cultures. No spores found. In old cultures the rodlets are so short as to be almost cocci.
Gelatine-plates.	Visible in about three days at 15–20° C. At first small, milk-white, opaque, circular colonies, growing very slowly. Under the microscope they are yellowish, granular, and sometimes faintly zoned; on emerging to the surface they spread irregularly in very thin sheets, with notched margins. May be rugose and contoured like Typhoid. Violet pigment begins to show faintly about the tenth day, and the gelatine is softening and liquefying immediately

around by this time. Liquefaction may be complete, or soon arrested. Submerged colonies may remain yellowish, or only become faintly coloured: the exposed ones become deep blue-violet, and all stages to white may occur on the same plate.

Streak-culture on gelatine. At 15° C. forms a white, opaque, milky streak in 24–48 hours. In three days may show signs of sinking as gelatine softens, and violet hue may begin to appear along the axis. In nine or ten days the gelatine is scooped, and a violet membrane floats on the liquefied mass, in which violet and dirty-white flecks are distributed. More or less complete liquefaction of the gelatine follows, but it often requires many weeks, even at 20° C., for completion throughout the tube.

Stab-culture in gelatine. In three days at 15° C. a small white button, like a drop of milk, is formed at the point of inoculation, and minute white points in the tunnel. In ten days or so the button has sunk in a depression of liquefied gelatine, producing a thistle-head funnel. Culture still white. In eighteen days the liquefaction reaches nearly to the walls of the tube, the funnel being lined by a violet membrane. In about four weeks the gelatine is liquefied to one-eighth of its depth or so, a deep violet, folded membrane floating on the top and through the liquid. At 20° C. the same phenomena are observed, but proceed more rapidly at first. It takes many weeks or even months to completely liquefy all the gelatine. Submerged in gelatine: no growth.

Agar. Forms a thick white streak in 24–48 hours at 20° C. which turns violet along the axis on the fourth day or later. In about ten days a magnificent corrugated, deep violet membrane is formed, which can be lifted, and shows white growth below. When water of condensation is collected, the submerged growth is white.

Potato. In from two to five days, at 20° C., a more or less copious, dirty-white to yellowish patch. Later on the violet colour spreads from the centre, and a corrugated, deep violet membrane is found by the tenth day. But this often does not extend to the edges, and in some cases fails altogether.

Broth. Traces of turbidity in two days at 20° C., and by the sixth day very turbid, especially towards the top, where a thick white membrane forms. After ten or twelve days, or even earlier, the thicker membrane begins to turn violet and a white precipitate falls in the still turbid liquid, in which are violet and white flecks.

Milk. The casein is precipitated as a coagulum, and slowly dissolved. The liquid is alkaline. Later on a violet membrane forms above.

Glucose. No fermentation, and no perceptible change at all beyond a very faint turbidity during the first few days.

Air requirements. Aërobic.

Temperature.	The Bacilli are still alive after several days at 35° C., but no growth is maintained; growth occurs at 5° C. The best temperature seems to be near 20° C. Old cultures withstand 50° to 60° C. for some hours, though no true spores are known.
Liquefaction.	Liquefies the gelatine, but only slowly after the upper parts are fluid, and the deeper parts may be solid months afterwards.
Rapidity of growth.	Slow. In many cases colonies are found only 10 or 12 mm. in diameter six months after making a plate, the gelatine around having dried up; they are usually about half that diameter when the gelatine liquefies, and they float in it.
Pigment.	Deep violet. Not in the cells, and does not diffuse into the solid media, except perhaps a little on potatoes. Insoluble in water; very soluble in alcohol, and looks like gentian-violet; very stable except in sunlight. Turns bluish green on adding NaHO, the original colour almost restored by excess of acid. Acetic acid to original solution makes it paler. Absorbs yellow-orange to green-blue.
Reactions to light.	Easily killed on exposure to direct sunlight. At moderately high temperatures the Bacilli, quiescent in darkness, move actively when illuminated.
Pathogenicity.	Prof. Kanthack finds it not pathogenic for guinea-pigs. I have to thank Prof. Kanthack for examining pathologically a large number of these Thames Schizomycetes.

Interesting and important results were obtained on reviving No. 2 from an agar-tube which had remained untouched from August 15 to June 9, i. e. ten months.

The plate-colonies at 20-22° C. eventually came up white and quite normal, except that they were more tough and membranous than expected, and the needle lifted each colony whole from its liquefying disc. At first they were pure white, as usual; but in ten days the purple colour appeared in the middle or at the margins, and rapidly spread. Until the purple hue appeared, I was inclined to suspect the plates were not pure, and that the colonies were those of a capsuled Bacillus.

The tube-cultures were also normal, and the purple membranes of the broth- and milk-cultures appeared on the tenth to eleventh days at 20–27° C., so that no doubt need exist as to the species. At the same time it should be noted that some of the plate-colonies remained white or yellowish-white to the end, and that great variations were exhibited as to the degree of liquefaction and coherence of the colonies. I was

unable to determine the causes here at work, but neither temperature nor impurity of culture were among them.

It is evident that this is a well-marked Pigment-Bacterium, and at first sight one might suppose it not difficult to identify. On looking further into the matter, however, it turns out that several violet or deep blue Pigment-Bacteria are described, some of them very vaguely. So far as I have been able to discover, the following are the forms hitherto introduced into the literature. Passing over Schröter's *Micrococcus violaceus*[1], a form described as not liquefying the gelatine, and non-motile —though in other respects it would seem to be very similar to the elliptical cocci got in old gelatine-cultures of the Thames species, and in any case it must be regarded as imperfectly described—there are at least nine or ten alleged specific forms in the handbooks, viz. *B. violaceus* of Plagge and Proskauer, usually regarded as identical with their *B. lividus*[2]; *B. janthinum* of Zopf[3]; *B. membranaceus amethystinus* of Eisenberg[4]; *B. violaceus Laurentius* of Jordan[5]; *B. violaceus* of Frankland[6]; *B. coeruleus*, Smith[7]; *B. coeruleus* of Voges[8]; *B. violaceus* of Macé[9]; *B. berolinsis indicus* of Claessen[10]; and *B. indigoferus* of Voges[11].

Assuming these to be all autonomous forms—which, however, is extremely doubtful—I now proceed to examine their principal characters with reference to the Thames form. Frankland's species—*B. violaceus*—naturally suggests itself, seeing that it was also reported from the London as well as the Berlin waters; but it is noticeable that it was first observed in the Spree.

Apart from small differences as regards size and manner

[1] Schröter in Cohn's Beiträge, Bd. i, p. 109; and Adametz, Die Bakterien der Nutz- und Trinkwässer, Wien, 1888.
[2] Zeitschr. f. Hygiene, Bd. ii, p. 463.
[3] Die Spaltpilze, 1885, p. 68.
[4] Bakteriol. Diagnostik, 1891, p. 421.
[5] Report of State Board of Health, Massachusetts, 1890, p. 838.
[6] Zeitschr. f. Hygiene, Bd. vi, p. 394.
[7] Medical News, 1887, p. 758.
[8] Centralbl. f. Bakt., Bd. xiv, 1893, p. 303.
[9] Annales d'hygiène publ. &c., t. xvii, 1887.
[10] Centralbl. f. Bakt., Bd. vii, p. 13.
[11] Ibid., Bd. xiv, 1893, p. 307.

of movement, the morphological characters of Frankland's species agree very well with mine, except that I have not observed the spores described as occurring in the agar-cultures. It is true that Frankland says they only occur here and there; but since they bulge out the Bacillus, they ought to be easily seen. However, the authors give no further particulars about these spores, and so it is impossible to form any opinion concerning them. In many respects, also, there are resemblances between the Franklands' form and mine in the characters of the plate- and other colonies; but they do not refer to the zoogloea-membrane, and describe the agar-cultures as forming a smooth, bright layer over the surface, and not a corrugated membrane.

Perhaps the sum of the differences gives us sufficient characters to separate the two forms, and we may say that mine differs from the Franklands' form in certain morphological characters: in forming no spores, in the marked development of zoogloea-membranes, and in the cultures on agar and potato. It might possibly be that the smooth, bright layer referred to by the Franklands is a young stage, or that they were working with a weaker form. I often find that after being some months in culture this form grows feebly on agar and potato.

The *B. violaceus Laurentius* of Jordan agrees fairly well in size [1], in forming no spores, and in some other morphological characters. The plate-colonies are very different, as are also the stab-cultures—especially as the author insists that no membrane forms above, and the gelatine rapidly liquefies in the tunnel. The cultures in milk and in broth are also markedly different.

The *B. violaceus* of Macé is believed by Macé himself [2] to be the same as the *Micrococcus violaceus* of Schroter, and the one found by Bujwid in hail [3].

[1] I assume that the breadth is 0·7 μ and not 7 μ, as stated in the original l. c. p. 838, obviously by a printer's error.

[2] Macé, Traité pratique de Bactériologie, 1892, p. 541.

[3] Bujwid in Ann. Pasteur Inst., 1887, p. 592.

Zopf's *Bacterium janthinum* differs in some characters—size, milk-cultures, &c.—but might very well pass for a feeble form of mine in other respects. Lustig[1] regards Zopf's form as identical with the one found by Bujwid in hail, and agrees with Jordan[2] that it is the same as one previously found by Hueppe. Jordan also points out the resemblances between Zopf's form and Rosenberg's *Bacterium h.*[3], as well as the doubts as to what the latter is—possibly identical with Frankland's *B. violaceus* or with Jordan's *B. violaceus Laurentius*. Zopf's *B. janthinum* has been found often in water, e.g. by Plagge and Proskauer[4], Roszahegyi[5], Jordan[6], &c. The latter also discusses the difference between the three forms last mentioned.

As regards Smith's *B. coeruleus*, from the Schuylkill, if the colouring-matter is really formed in the cells, as stated, and is insoluble in alcohol, there is no doubt a sufficient difference; moreover, the pigment is a *blue* one, and we may safely regard this as a distinct form on the evidence to hand.

Claessens' *B. berolinsis indicus*, from the Spree, also produces a blue (indigo) pigment, insoluble in alcohol, and many other differences exist, in addition to its not liquefying the gelatine and not rendering broth turbid[7], which suffice to distinguish it.

Voges' *B. coeruleus* is too stout and short for my form, and again the pigment is a different colour—blue, not violet—and does not form in broth. The agar- and milk-cultures are also decidedly different. Moreover, the colour is soluble in water. There can be no doubt this form is quite different from the one I have isolated from the Thames.

Voges' *B. indigoferus* is still more different, and especially in its minute size.

There remains Eisenberg's *B. membranaceus amethystinus*. The differences between this form and mine are so few and

[1] Diagnostik der Bakterien des Wassers, 1893, p. 76. [2] l. c., p. 841.
[3] Ueber die Bakterien des Main-wassers, Arch. f. Hyg., Bd. v, p. 458.
[4] Zeitschr. f. Hyg., Bd. ii, p. 458. [5] Lustig, l. c., p. 76.
[6] l. c., p. 840. [7] According to Voges, l. c., p. 302.

so trivial that I am strongly inclined to regard them as accidental. Eisenberg's description of the plate- and other gelatine-cultures, of the development of the membrane on agar and broth, absence of spores, and so on, are quite like mine; but the rods are extremely short, and non-motile, though their thickness agrees very well. His description of the potato-cultures also agree with some of mine, though in other cases I find the pigment developed on these also.

On the whole, it seems pretty certain that this violet Bacterium from the Thames is, then, the one found by Jolles in water, and named *B. membranaceus amethystinus* by Eisenberg.

Other Pigment-Bacteria, with blue or violet hues, are referred to in the literature, but the descriptions are so incomplete that I cannot compare them. Thus Schröter's *B. Lacmus*[1], Ehrenberg's *B. syncyanus*[2], Beyerinck's *B. cyaneofuscus*[3], forms a blue pigment in one of its stages, but does not properly come under the head of violet Bacteria. What Jordan's *B. cyanogenus*[4] is, and whether it is the same as Hueppe's[5] Bacillus of blue milk, I cannot decide, but both may safely be regarded as distinct from any of the forms referred to, and the pigment is quite different. The same is true of Alvarez' *B. indigogenus*[6], and so far as I can discover these exhaust the list of blue and violet Pigment-Bacteria.

It not unfrequently happens that plate-colonies refuse to colour. I have made numerous attempts to determine the cause of this. Exposure to light, and cultivation at too high a temperature, may bring it about, and in one series I had it in culture for nearly a year as a white or slightly yellowish form which refused to form the violet pigment. Eventually, however, the colour appeared in a milk-culture, and was

[1] Pilz. Schles., p. 158, referred to by De Toni and Trevisan in Saccardo's Syll., Vol. viii, p. 978.

[2] Verhandl. d. Berl. Akad., 1840, p. 202, and Saccardo, l. c., p. 979.

[3] Bot. Zeit., 1891, No. 43.

[4] Jordan, l. c., p. 832.

[5] Mitth. aus dem Kaiserl. Ges., Bd. ii, p. 355 (see also Heim, *ibid.*, Bd. v, p. 518).

[6] Sternberg, Bacteriology, 1893, p. 476.

regained more and more on transference. In some cases, however, the non-pigmented variety appeared on normal plates, and I was quite unable to refer it to any cause. This white variety would probably repay further study.

In any case I think the evidence is against the multiplicity of species of violet Bacteria which now exist in the literature. At the same time, the difficulties of microscopic cultures of the present form, and the lack of information regarding them in other alleged 'species' or forms, should make us hesitate before we decide as to the autonomy of any, since it may be taken as certain that we do not know the whole life-cycle of even a single member of this type.

EXPLANATION OF FIGURES IN PLATE VI.

Illustrating Professor Ward's paper on a violet Bacillus.

Fig. 1. Rodlets from fresh gelatine (*a*) not stained; (*b*) similar rodlets stained with methylene-blue—the ends often stain more deeply than the centre.

Fig. 2. Rodlets, &c., from an old Agar culture (*a*) stained with methyl-violet; (*b*) similar preparation from old gelatine-cultures showing 'involution-forms.'

Fig. 3. Rods and filaments from a twenty-four hours' broth-culture.

Fig. 4. Plate-colonies on gelatine after ten days' growth at 20° C., from a culture a year old (nat. size).

Fig. 5. Plate-colonies in various stages of development on gelatine: (*a*) after twenty-four hours at 18° C.; (*b*) the same on third day; (*c*) submerged colonies on fourth day at 18° C. All under $\frac{1}{5}$ obj.

Fig. 6. Plate-colonies: (*a*) after ten days at 18° C., nat. size, showing development of pigment, and liquefaction; (*b*) the non-pigmented variety after three weeks at 18° C.; the plate is liquefied, and cream-coloured or yellowish colonies are floating in the liquid.

Fig. 7. Stab-cultures in gelatine: (*a*) after three days; (*b*) after ten days, liquefaction is beginning, but the colonies are still white; (*c*) after eighteen days, the violet pigment appearing in the funnel; (*d*) after a month, the gelatine liquefied some way down. All at ordinary temperatures.

Fig. 8. Gelatine-streak after ten days at 15° C.; the purple growth lies in a groove of liquefaction.

Fig. 9. Agar-culture at 20° C.; (*a*) after four days, the purple colour appearing in the white, and (*b*) on the tenth day, the white nearly all gone.

Fig. 10. Potato-culture, ten days at 20° C., the purple hue has only invaded part of the growth—the rest remains dirty brown in colour.

Fig. 1.

Fig. 3.

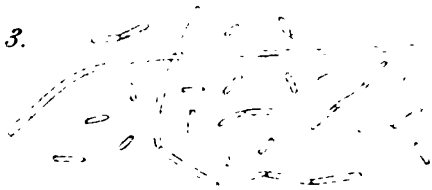


Fig. 4.

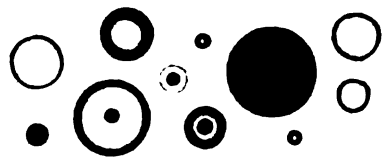


Fig. 2.

Fig. 5.

Fig. 6.

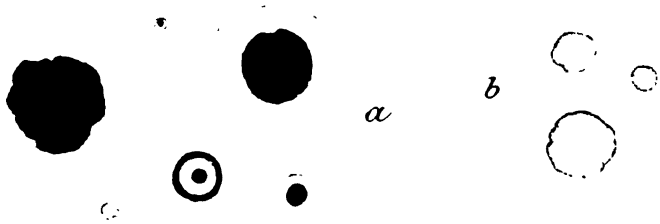


Fig. 7.

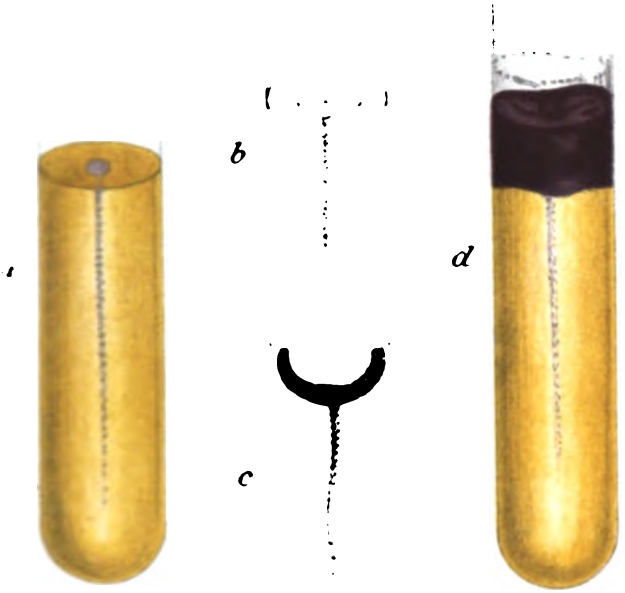


Fig. 8.

Fig. 9

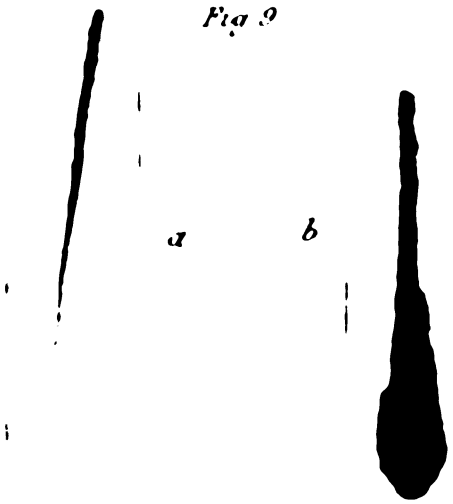


Fig 10.

The Polymorphy of Cutleria multifida (Grev.).

BY

ARTHUR H. CHURCH, B.A., B.Sc.,

Jesus College, Oxford: Demonstrator of Botany in the University.

With Plates VII, VIII, and IX.

SINCE the classical researches of Falkenberg[1], carried out at Naples in 1878, in which he showed the necessity of fertilization for the oospheres of *Cutleria multifida* (Grev.), and established the identity of the product of germination of such sexually-produced spores with *Aglaozonia reptans* (Kutz.), but little has been done to clear up the mystery underlying his conclusion that *Cutleria* must therefore present an antithetic alternation of generations, in which *Cutleria multifida* represents the gametophyte, while *Aglaozonia reptans* is to be regarded as a true sporophyte-generation.

Conclusive evidence of such an alternation of generations would be of greatest theoretical interest; not so much from its being the only example described in the Phaeophyceae of an antithetic alternation at all comparable with the phenomena occurring in the Coleochaetaceae and the Florideae,

[1] Die Befruchtung und der Generationswechsel von Cutleria: Mittheilungen aus der Zoolog. Stat. zu Neapel, vol. i. 1879.

[Annals of Botany, Vol. XII. No. XLV. March, 1898.]

which are regarded as possibly representing evolutions of alternation, in existing groups of Green and Red Algae, parallel to that which has reached such a degree of complexity in the Archegoniatae; as from its presenting, within the limits of a very narrow alliance, a sudden transition to a true alternation from the simpler life-history of the homotype genus *Zanardinia*.

Falkenberg showed, beyond all doubt, that *Aglaozonia* plants were normally the ultimate product of sexual reproduction in *Cutleria multifida*; but, owing to the death of his plants, the question as to the subsequent relations of the *Aglaozonia* to *Cutleria*—whether the latter arose from spores, or was the result of merely vegetative reproductive processes—was left open; as was also that of the strictness of the alternation on the side of the perennial and more widely distributed *Aglaozonia*.

Although the following observations, made at the Marine Biological Laboratory at Plymouth, cannot be regarded as finally solving the problem, it is hoped that they may contribute to a more complete acquaintance with the life-history of these plants.

Both *Cutleria* and *Aglaozonia* grow in the estuary of the river Yealm, near Plymouth, at 2–3 fathoms below low-water mark, and may easily be obtained, at all states of the tide, by dredging. *Cutleria* grows as a summer annual, reaching its maximum development in July and the beginning of August. It rapidly diminishes in quantity in September, and has completely disappeared by October. *Aglaozonia*, on the other hand, is perennial, growing on stones and shells, especially oyster-shells, in the same locality and depth, reaching its finest development in October and November. Poor in quality during the winter-months, possibly owing to its being eaten by Mollusca &c. in the absence of other vegetation, it recovers in the spring, and bears reproductive sori in March and April. It is also usually in poor condition throughout the summer. From these data, it would appear, therefore, that in English waters *Cutleria* is a rapidly-

developing summer sexual plant, *Aglaozonia* a slow-growing perennating winter form; and that these two growth-forms had become complementary in structure and habit, as well as in reproductive functions.

A consideration of the conditions under which these two plants live in the Bay of Naples shows, however, that this does not represent the whole truth. The majority of the most interesting of the summer annuals growing in shallow water on the southern shores of England are, in the Bay of Naples, early spring- and even winter-plants; while others, on the other hand, retire to deeper water. A few examples will make this clear: thus, *Cutleria* at Plymouth grows in company with abundant *Sporochnus*, *Arthrocladia*, *Stilophora*, *Asperococcus bullosus*, *Dictyota*, and such Florideae as *Dudresnaya coccinea* and *Scinaia furcellata*; all these forms reproducing freely in July and the beginning of August, and growing in 2–3 fathoms of water at a temperature of 18 C. From data given by Falkenberg for the Bay of Naples, it appears that *Sporochnus* and *Arthrocladia* flourish also there in July and August, but at a depth of 20 fathoms; *Stilophora* follows the *Cystoseira* zone at a slightly less depth and grows in early summer; *Asperococcus bullosus* also in spring and summer, varying from 1–8 fathoms; *Dudresnaya* at 2 fathoms in March and May; and *Scinaia* at the same depth from February to June, although the latter has been dredged at Messina in July at 15 fathoms.

Of the plants with which *Cutleria* grows in English waters, therefore, some retain at Naples the same annual period, but live in far deeper water; but more generally the depth of water remains fairly constant, while the season of the year is changed. There can be little doubt that the two determining factors of external environment are temperature and intensity of sunlight, with the latter being associated a greater degree of purity in the water, which in the Bay of Naples allows vegetation to flourish as far out as 40 fathoms, while, in the immediate vicinity of Plymouth Sound, only scanty traces are met with at even 10 fathoms. *Cutleria*

at Naples follows the rule of the majority of its English associates and vegetates in shallow water from December to April, vanishing, like *Dictyota* in the Mediterranean, on the approach of summer. It is important to note that while *Aglaozonia* is also perennial in the Bay of Naples, *Cutleria* is the winter-form, completely disappearing by April, its existence being apparently terminated by a rise of temperature, instead of by a fall as on the English coasts. It is clear, therefore, that the vital capacities of the sexual plant towards temperature are much more limited than those of the asexual *Aglaozonia*, which is perennial, not only in the warmer waters of the Mediterranean summer, but in the cold waters of the North Atlantic and North Sea winter.

If now we compare the geographical distribution of the known species of the Cutleriaceae[1], we find that the order belongs naturally to the warmer seas. Thus, omitting the doubtful *C. Laminaria*, Kutz. of the Mediterranean, the group consists of two little known species, *C. pacifica* from Samoa, and *C. compressa* from La Guayra; of *C. adspersa* of the Mediterranean district only[2] (Cadiz to Suez); of *Zanardinia collaris*, Mediterranean, West Indies, Polynesia, the Atlantic shores of Europe as far as Brest (Crouan), drifted specimens at Jersey (Harvey); and of *C. multifida*, also a Mediterranean and Atlantic type[3]. But this last, alone of the group, extends northwards to England, Shetland Islands, and the coast of Norway to Nordland (Kjellman); on the other hand, it is poorly represented in the North Sea district and absent in the Baltic. That is to say, the northward distribution of the sexual form appears to be limited

[1] De Toni, Sylloge Algarum, vol. iii. p. 300.

[2] Sauvageau (Journ. de Bot. 1897, p. 177) since gives *C. adspersa* and an undetermined *Aglaonia*, but neither *C. multifida* nor *Zanardinia*, as being abundant in winter, at low-tide mark, in the Gulf of Gascony; and Mr. Batters informs me that *C. adspersa* is found at Brest.

[3] A doubtful Polynesian form of *Aglaozonia* described as *Zonaria parvula* var. *duplex* (Heydr. Beitr. Algenfl. v. Kais. Wilh. Land), placed by De Toni under *C. multifida*, might more possibly belong to *C. pacifica*.

by the temperature of the northern summer, and in the English Channel we are already beyond the natural home of the *Cutleria* family.

Parthenogenesis of Cutleria.

The first recorded specimen of *C. multifida* was picked up after a storm on Yarmouth beach by Dawson Turner on August 31, 1804[1]. It was a female plant, covered with oogonia as the date would suggest, and was described in Smith and Sowerby's English Botany in 1805, under the name of *Ulva multifida* (No. 1913). It appears as *Zonaria multifida* in Agardh's Sp. Alg. 1824, and as *Sporochnus multifidus* in Sprengel's Systema Vegetabilium of Linnaeus, in 1825; it received its modern title in 1830, from Greville[2], who formed for it a new genus, named in honour of Miss Cutler of Sidmouth. Greville, also, knew only the female, or as it was considered, the sporangiate plant. Antheridial plants were described later by Dickie, but these were very rare, and Harvey in his Phycologia Britannica (1846) mentions that he had never seen more than one such plant, which had been sent him from Sidmouth.

At this time no sexual significance had been attributed to the reproductive cells of Algae, or these antheridia might have been a source of difficulty; but they were commonly regarded as imperfectly formed swarming cells which were consequently destined to remain sterile[3]. The first definite statements with regard to the emission and germination of the spores were made by Thuret[4] in 1850. He observed the discharge of the oospheres in the early hours of the morning, as also their active movement and strong positive heliotropism by means of which they rose to the surface of the water. In all cases germination was direct; the

[1] Harvey, Phycologia Britannica, t. 33.
[2] Algae Britannicae, p. 60.
[3] Cp. Nageli, Bot. Zeit. 1849, p. 569. [4] Ann. Sci. Nat. III. 14, p. 32.

oospheres came to rest, the pointed end grew out to form a rhizoid, the body of the spore giving a brown filament of a few cells. A number of female plants, kept in a vessel of sea-water, continued to give off oospheres for several successive days, which in all cases germinated perfectly without the admission of antherozoids. Thus, although Thuret was fully satisfied as to the necessity of antherozoids for the fertilization of the oospheres of *Fucus*, he concluded that no act of fertilization took place in the *Cutleria* spores he had under observation. It is also of special interest to note that he found antheridial specimens to be extremely rare at Saint Vaast-la-Hogue, where these researches were conducted; he states that he often collected from the oyster-beds there, where *Cutleria* grew in profusion, over a hundred female specimens before finding one male; and he points out that this rarity of antheridial specimens not only agrees with what Harvey had stated to be the case in English waters, but would to a certain extent militate against the view that antherozoids possessed sexual functions of such importance to the plant.

In the summer of 1855, the brothers Crouan[1] repeated these observations at Brest, and came to identical conclusions with regard to the perfect parthenogenesis of the oospheres. At the same time, they noted a peculiar phenomenon in connexion with the fate of the antherozoids. These at first rose to the surface of the water, forming an orange film on the side nearest the light, in the manner typical for all swarming cells of the Brown Seaweeds; but on coming to rest, they became agglutinated by their gelatinous membranes into a pseudo-tissue mass of a brown colour, which was even capable of being sectioned. They therefore concluded that the antherozoids were non-sexual, but still possessed a certain degree of germinative capacity. It is so far clear that to the older observers who worked on the French shores of the Channel, the constancy of the germination of the oospheres

[1] Bull. Soc. Bot. France, ii. p. 644.

was so apparent that the question of the non-sexuality of *Cutleria* was never in doubt.

The converse was however asserted by Reinke[1] in carrying out his researches at the Naples Station in 1875–76. He confirmed Crouan's observations on the peculiar pseudo-tissue formation of the antherozoids, but attributed to it no real germinative significance, since in all his experiments, antherozoids and oospheres, isolated from each other, constantly underwent no further development. On the other hand, in vessels containing both male and female plants, germination took place freely, and actual fertilization by the antherozoids was observed. From these facts he deduced the perfect sexuality of *C. multifida* and the essential importance of the antherozoids; as also that Thuret's observations must have been due to an accidental parthenogensis. It is interesting to note that he gives male and female plants as occurring in the Bay of Naples in the ratio of three male to two female.

Similarly Falkenberg[2], in 1878, described male and female plants as being about equally abundant in the Bay of Naples, and carrying out his experiments with great care in obtaining pure cultures of emitted oospheres and antherozoids, he fully confirmed Reinke's results. Moreover, as his cultures were free from extraneous growths of Diatoms, &c., which had ultimately induced pathological conditions in Reinke's cultures, Falkenberg succeeded in developing the germinated embryos to a considerable size. In all his experiments, antherozoids became immotile and useless in twenty-four hours, and then died; oospheres retained the capacity for fertilization for four or five days, but never commenced segmentation; fertilized oospheres germinated directly and rapidly; while unfertilized oospheres never got beyond the formation of a thin cell-membrane.

Further, Janczewski[3], at Antibes in 1883, showed in the case of *C. adspersa*, which is also a Mediterranean spring-plant,

[1] Nova Acta der K. L. C. Deutsch. Akad. xl. 1878.
[2] Loc. cit. [3] Ann. Sci. Nat. vi. 16. p. 210.

that neither male nor female sexual cells present any of these curious suggestions of direct development, but that both oospheres and antherozoids die the same day if copulation does not take place.

During the summer of 1896, male plants were extremely rare at Plymouth, only two or three being seen, although female plants were dredged in considerable quantity. In 1897 the same proportion obtained: thus, in a dredging taken on August 11, a score or so of very fine female plants were collected, but only one male. Some of these were placed in a vessel of filtered water with the object of obtaining embryos, but owing to the heat or some other cause, the plants all died; nor was it until the end of the month, when cold and wet weather set in, that freshly dredged material could be kept alive more than a day or two. At this time and onwards, all the plants obtained were female, no more male plants being seen for the year. With the object of testing Thuret's observations on direct germination, a number of female plants were on August 20 placed in filtered water (temp. 18° C.) standing in a north window. In the course of three weeks, the water having only once been changed, the fronds were found to be sprinkled all over with innumerable young plants, which by September 16 presented unmistakable *Aglaozonia* characters (Figs. 14–21). Although the immediate proximity of such numbers of these young plants to the sori of oogonia suggested at once the direct germination of oospheres which had lost their motility soon after discharge, it was quite possible that fertilization might have taken place before collection. More plants were accordingly collected in September, and washed and placed in filtered water. In a week the surface of the vessel was covered with thousands of germinating oospheres which had risen to the surface in virtue of their strong positive heliotropism; of these, the majority at least must have been parthenogenetic, as it is evident that any few antherozoids, which might have survived collection and washing on the female plants, would not have sufficed for such a multitude of oospheres.

On adding a fresh supply of filtered water, another week gave a second similar crop of germinating oospheres, and a third week yet another, thus confirming Thuret's original observations.

Several distinct cultures, some containing fragments of female thallus, still producing oospheres, which had been growing in filtered water in the laboratory since August, others containing freshly discharged oospheres only, were made towards the end of September. In all cases germination proceeded directly and quite normally, although slower than in the case of the first crop obtained in August, and far slower than in the experiments of Falkenberg, who states that his fertilized oospheres produced a plant of 3–4 cells in the first twenty-four hours, whereas the Plymouth cultures in September did not do more than this in the first week. It is probable, however, that this rate of growth varies directly with the temperature.

Finally, separate cultures, from small pieces of female plants collected on September 21, were made on October 12, and brought to Oxford and kept in a sunny window. In all cases germination again took place normally; in three days sufficient oospheres had collected on the side nearest the light to form a visible film. The majority of the oospheres were covered with a well-marked membrane and many had already put out the first rhizoid. The temperature was low (14) and the weather dull, but after two days of bright sunshine the plants increased to about five cells and a long rhizoid, and by October 25 they formed well-grown embryos in which segmentation was rapidly proceeding (Fig. 13).

At the end of three weeks (November 1), the culture-vessels having latterly been standing in bright sun for a few hours every day, an immense number of young plants in all stages of development were to be seen, the small piece of thallus in the culture continuing to give off oospheres. The oldest plants showed the 'foot-embryo' now at its maximum size, but with so far no formation of dorsiventral lobes (Fig. 14). That is to say, the germination of these unmistakably

parthenogenetic spores had proceeded at a rate equal to and with results in a given time identical with those observed in the first culture of August 11, and in which the possibility had not been eliminated that fertilized oospheres might have already become attached to the plants before they were gathered. That oospheres did this in the natural state was observed on specimens dredged in September, but it is clear that continued crops of free-swimming oospheres, germinating at the surface, were beyond suspicion. It is also of interest to note that the old plants which continued to give these crops of germinating oospheres had been, since the middle of September, in a rapid state of disintegration, and by November 1 were but partial skeletons compared with the perfect summer-plants; nor, at this time, would they have been found by dredging. No *Cutleria* was dredged at Plymouth in 1896–97 after the middle of September, the plants then evidently decaying and easily losing their point of attachment.

The general result of these observations, therefore, is not only to confirm the original observations of Thuret and Crouan, made on the opposite shores of the Channel, as to the absolute constancy of parthenogenetic development of the oospheres at the end of the summer; but, bearing in mind the equal constancy of fertilization observed by Reinke and Falkenberg in early spring at Naples, it further leads us to correlate the apparent contradiction of these observations with the fact that the conditions of external environment are so widely different in the case of plants growing in the Channel and in the Bay of Naples respectively; and further to suggest that the parthenogenesis of the Channel plants may be due to the fall of the temperature of the sea at the end of the northern summer, which, by diminishing the sexuality of the oospheres, causes the plant to become an asexual form by degeneracy, although morphologically retaining the distinction of sex.

Germination of the Oospheres.

In all cases, whether in later undoubtedly parthenogenetic cultures or in the earlier ones only doubtfully so, germination proceeded along lines absolutely identical with those described by Falkenberg for the sexually-produced spore.

The spore secretes a cell-membrane, becomes pear-shaped, and divides into a shoot-cell and a first rhizoid (Fig. 11); the latter elongates and reaches a considerable size if germination takes place at the surface of the water, but remains short on contact with any foreign body. The shoot-portion of the plant gives rise to a filament of a few cells only (6–10) by intercalary rather than apical segmentation (Fig. 12), and then definitely ceases to elongate; this being, according both to Falkenberg's and the Plymouth experiments, all that remains in this type of germination to mark the primitive filamentous condition of the *Cutleria* (Fig. 12, one week old).

Irregular segmentation commences immediately throughout the young plant; any and ultimately every cell dividing repeatedly by walls in different quadrant-planes, until the embryo becomes a more or less club-shaped multicellular mass of tissue, attached by one extremity and still exhibiting radial symmetry (Figs. 12, 13, 14).

To this stage Falkenberg has given the name of the 'Foot,' and it is probably representative, both phylogenetically and ontogenetically, of a primitive thalloid condition in which the main axis of the plant was radially symmetrical and segmented behind the growing region in the regular manner seen in such a form as *Stypocaulon*. When well-developed, the foot may form a well-marked tissue-mass (Fig. 14); but it is often, and this was more general in some cultures than others, to a great extent abbreviated in development, ultimately giving rise to an embryo which was practically dorsiventral throughout (Fig. 16), and identical with the oldest embryos obtained by Janczewski[1] in *C. adspersa*.

[1] Loc. cit. p. 220.

At one or more points in the 'Foot,' any single superficial cell may initiate a new growth (Fig. 15), which, by successive T-shaped walls, gives a lobed outgrowth which exhibits dorsiventral symmetry, and by laying down the marginal segment-walls preferentially in a radial vertical plane, assumes a fan-shaped outline, the commencement of an *Aglaozonia* disc (Figs. 15, 19, 20). The formation of these lobes appears to be mainly due to the stimulus of contact, and thus a majority form discs at the point of attachment (Figs. 19, 22); but if the apex of the 'Foot' bends over, a symmetrical outgrowth may take place there, either alone or in addition to another at the base (Figs. 17, 18, 20). In the case of the foot lying more or less prostrate, several (6–8) distinct lobes may be produced, which develop rhizoids on the side towards the substratum (Figs. 20, 21). In plants which have become detached, the dorsiventral lobes continue to be formed and exhibit a tendency to curl up, indicating a return to radial symmetry comparable to that of the proliferating 'cups' of *Zanardinia*.

Although many distinct cultures were made, and hundreds of embryos observed, in no single case was any further development noticed in the 'Foot'; the dorsiventral lobes slowly but steadily increasing along definite *Aglaozonia* lines.

Following Falkenberg, this type of plant may suitably be distinguished as the *Foot-Embryo*.

It will therefore be noted not only that these observations on the development of the *Aglaozonia*-thallus from oospheres of *Cutleria* absolutely confirm those of Falkenberg, but that such confirmation was necessary, since the embryos observed by Thuret[1] at Saint Vaast-la-Hogue in 1850 were unmistakably different: it was in fact the figure given by Thuret of a free-growing filament of thirty-six cells with branches towards the base, which appeared, as being a vegetative growth homologous with an adventitious branch, to confirm his assertion of the non-sexuality of the oospheres.

[1] Études Phycologiques, and Ann. Sci. Nat. iii. 14.

Not only were the Plymouth plants truly parthenogenetic, as opposed to Falkenberg's truly fertilized ones, but they were grown in the autumn months, whereas Falkenberg's were grown in the spring; the only factor in common therefore appears to be this, that in either case the spores were obtained from mature plants about to die, from summer-heat in the latter case, but from winter-cold in the former.

Germination of Zoospores of Aglaozonia.

Aglaozonia plants were first described by Greville[1] in 1828, from specimens found, appropriately enough, by Miss Cutler at Sidmouth, growing at low-tide mark on exposed sand-stone rocks; these sterile plants being placed as a new species in the genus *Zonaria* of C. Agardh under the name *Zonaria parvula*. Later Greville[2] founded a new genus, and changed the name to *Padina parvula*; and in 1833 similar sterile plants found by Crouan[3] at Brest were distinguished as *Padina reptans*. Reproductive organs were first found on Skagerack specimens by Areschoug in 1843, and the genus refounded as *Padinella*. Areschoug's plants were very small, and possibly dead before examination, as his figures[4] are quite misleading[5].

The genus *Aglaozonia* was ultimately established by

[1] Crypt. Flora, t. 360.
[2] Alg. Brit. 1830, p. 63.
[3] Florule du Finisterre, p. 169.
[4] Linnaea, 1843, p. 260.
[5] Areschoug obtained his plants on oyster-shells at Koster, and was satisfied that they were identical with Sidmouth specimens described by Greville. His drawing appears to have been made from a squeezed-out sorus, rather than from a section; and the appearance which it presented induced Reinke to revive the old name of *Zonaria parvula* for a plant he obtained at Naples in 1875 (Nova Acta, xl, No. 1. p. 34), which was of distinctly Dictyotacean nature. Reinke's plant differs fundamentally from *Aglaozonia* in the structure of the thallus, the well-marked 'tetraspore,' and, above all, in the embryology, which is again that of the Dictyotaceae. It is quite obvious that Greville's Sidmouth plants were *Aglaozonia*, as they still grow there abundantly, and Areschoug had received specimens from that locality; but it is not clear why Reinke's distinctly Dictyotoid plants should be classed as Cutleriacean by De Toni (Sylloge Algarum, Fucoideae, p. 234).

Zanardini [1], and Kützing, in his Species Algarum (1849), gives both *Aglaozonia parvula* for the English and Mediterranean plants, and *Aglaozonia reptans* for Crouan's specimens. The sporangia and the emission and asexuality of the zoospores were correctly described by the brothers Crouan [2], at Brest, in 1856, from the large quantities of material they found thrown up by a storm on April 5 of that year, while the first correct drawings were given by Zanardini [3] in 1860. Since then the plants have been known as *Aglaozonia reptans*, it being clear that Crouan's specimens were not only identical with those found elsewhere, but were the first on which the reproductive organs were definitely observed.

The discs of *Aglaozonia* are perennial, and are distributed from the Mediterranean along the Atlantic shores of Western Europe to the coast of Norway, being much more abundant and more widely distributed than is *Cutleria* along the Norwegian coast [4]. Again, they are more general than *Cutleria* in the North Sea, and are found abundantly in the more northern portion (Berwick) where *Cutleria* is unknown; and finally, they penetrate into the milder climate at the entrance of the Baltic, and are moderately common in the Skagerack [5], where *Cutleria* is very rare, or only found as very young specimens. *Aglaozonia* reproduces in the Mediterranean in late autumn, in the Channel in early spring, and it would appear that Areschoug found his Swedish specimens in reproduction during the summer months. On March 29, 1897, shells bearing fine plants of *Aglaozonia* with reproductive sori were dredged in the river Yealm, Plymouth. One or two sori were carefully removed, placed in a glass dish of filtered water, and allowed to stand in a window exposed to a north light. Zoospores were set free in great numbers, and rising to the surface, swam towards the side nearest the light, forming in a day or two

[1] Saggio di classificazione nat. delle Ficee, 1843.
[2] Bull. Soc. Bot. de France, 1857. [3] Icon. Phycolog. Adriatica.
[4] Kjellmann, Handbok Skand. Hafsalgflora, 1890, p. 17.
[5] Gran, Algenvegetationen i Tonsbergfjorden.

a distinct brown film. Germination, as already described by the brothers Crouan, took place immediately, and with considerable rapidity (Fig. 1). As in the case of the oospheres of *Cutleria*, the zoospores came to rest, the anterior end became attached to the sides of the vessel or to another plant, and grew out into the first rhizoid. In the case of free-floating spores the rhizoid elongated considerably if it did not come into contact with anything, but ceased to elongate further on contact (Fig. 2). A simple filament of 3–6 cells was formed in a few days, and this agrees with the rate of germination observed for the oospheres of *Cutleria* grown at an approximately equal temperature.

As growth proceeded, the film stretched over the surface of the water in the vessel, forming a pure culture of germinating spores, from which portions could be readily transferred to other vessels of water similarly filtered by a Berkefeldt filter. Beyond keeping the vessels covered, to prevent evaporation and the entry of dust, no further change was made; the best results, in fact, being obtained from the original culture in which the water remained unchanged for over a month.

The filaments continued to elongate, by intercalary rather than apical growth, but the characteristic cessation of growth observed in the foot-embryo of *Cutleria* did not set in; steady intercalary growth enabling the filaments to double their length each week (Figs. 5, 6).

In the second or third week, differentiation in the cells of the filament became marked. The cells in the basal region of the plant increased in bulk and commenced segmenting irregularly by walls in different planes, thus rendering a lower region of the embryo multicellular by the same quadrant-walls, and at about the same age, as in the segmentation of the foot-embryo (Figs. 5–9).

This basal multicellular region is therefore homologous with the foot itself, but the embryo differs in that the filamentous terminal portion goes on growing by a definite intercalary zone (Figs. 7–10).

A further exaggeration of the basal segmentation resulted in the formation of a small irregular attachment disc (Fig. 10); but the latter exhibited no immediate tendency to extend into dorsiventral lobes, the main energy of growth being, in this embryo, clearly localized in the filamentous portion. This continued to grow, throwing out branches above and rhizoid attachment-hairs below. In the case of plants growing on the sides of the vessel, the filaments showed a tendency to attach again at any point in their length, sending out rhizoids, and initiating a new intercalary zone of growth above each such attachment: but it is possible that this may be an abnormal result of cultivation, as the same tendency can be observed in cultures of old *Cutleria* plants, where the reproductive filaments elongate and attach themselves to the sides of the vessel by rhizoids and bear gametangia at irregular intervals; the filamentous portions of the adult *Cutleria* are, in fact, still in the condition of these filamentous embryos.

It will be seen that even these young plants present the majority of the essential characters of the *Cutleria*-thallus: there is, for example, the same intercalary growth of a filamentous apex, with irregular segmentation behind the growing-point leading to a multicellular condition, and the same throwing-out of branches of similar growth and of attachment-rhizoids to supplement the primitive holdfast. The only point lacking is the aggregation and fusion of the branches behind the growing-points to the peculiar fasciated thallus of the adult *Cutleria*.

At the beginning of May, a culture of these young plants, now a month old and forming tufts of actively assimilating filaments, was taken to Oxford and kept under observation in a shaded situation in a south window of the Botanical Laboratory. The plants continued to live and assimilate vigorously, forming a bright brown woolly growth of *Ectocarpus*-like filaments in the unchanged water, and maintaining their position at the surface in virtue of the gas-bubbles evolved. In still water, this phenomenon affords the surest

test of the health of a culture, death rapidly ensuing if the plants once sink and are unable to again raise themselves. In no case was any further advance made in the formation of the adult *Cutleria*-thallus; the filaments in some cases showed the rope-like aggregation characteristic of the main branches of many *Ectocarpus*-forms, but no fusions to a pseudo-tissue took place. The filamentous mass increased in bulk for over a month, but after that the plants began to be sickly, and by the end of June the whole culture was undoubtedly so, and portions of it commenced to die off. Before dying however, in July, the plants produced multilocular reproductive organs in great abundance throughout the culture, which, on maturity, proved to be unmistakable antheridia of *Cutleria* (Fig. 3).

In the same culture (the only one which reached this stage, for all the plants left at Plymouth died at an early date) many of the young plants had, in addition, thrown out *Aglaozonia*-lobes from their attachment discs, and some of these fully equalled in extent a two months' old *Aglaozonia* grown from *Cutleria*-oospheres (Fig. 4).

Although abnormal conditions may have led to pathological results, it was undoubtedly shown that *Aglaozonia*-zoospores, under certain conditions, not only give rise to a *Protonematoid stage of Cutleria*, which on impoverishment and exposure in a sunny window in summer became precociously antheridial, but that they may, on the other hand, produce the *Aglaozonia*-form again, and thus the antithetic character of the alternation would fail to be established.

As already indicated, these observations are still incomplete, since the observation of the development of the mature assimilating thallus of *Cutleria* has yet to be made; but this is not absolutely essential, since a filamentous plant bearing oogonia, but presenting even fewer of the vegetative characters of a *Cutleria*, in that it was almost a constantly uniseriate filament throughout, has already been described by Kuckuck under the name *C. multifida* var. *confervoides*[1].

[1] Wissenschaftliche Meeresuntersuchungen, Biolog. Anst. Helgoland, 1894, i. p. 251.

Kuckuck's plants came up spontaneously in the tanks of the Heligoland Laboratory in the summer of 1893, and grew as short filaments attached to stones which had been collected in the North Haven in fairly shallow water (1–3 fathoms). Similar plants were found in reproduction in July, forming brown *Elachista*-like tufts on *Plocamium*, and sterile plants also as late as December.

Normal *Cutleria* is said to have been gathered at Heligoland by Wollny, but has not been known to occur there since; and although Kuckuck appears to infer that his plants reproduced their like, it is quite probable that they had all sprung from *Aglaozonia*-spores, and owed their late and feeble development to the cold spring and early summer of the North Sea; and that thus unfavourable conditions had led to a vegetative degeneration similar to that observed in the Plymouth cultures.

But if they had been reproduced from oospheres similar to those they bore, the confirmation of the development of a protonematoid embryo from a *Cutleria*-form would be of still greater theoretical interest, as confirming Thuret's original observation, and thereby assisting in the demolition of the theory of inherent necessity of an alternation of growth-forms.

Seasonal Dimorphism.

From the preceding considerations it is obvious that the polymorphy of *Cutleria* presents little in common with the antithetic alternation of primitive gametophyte and nursed sporophyte of the Archegoniatae; and still less with the case of *Coleochaete* and the Florideae, in which the origin of what in these forms is generally regarded as a sporophyte may be sought in polyembryony.

From the homology of the *Aglaozonia*-thallus with other asexual Algae such as *Battersia* or even *Laminaria*, *Aglaozonia* has as much claim to be regarded as theoretically a gametophyte as any other Alga. It might even be urged,

that, as it has not only a wider geographical, but higher tide-mark distribution, in virtue of its greater power of resistance to extremes of temperature and wave-action, and is moreover perennial and capable of reproducing its like, *Aglaozonia* has even a better claim to be regarded as the most important of the two forms, and therefore more entitled to be regarded as the phylogenetic and theoretical gametophyte than the delicate, sexual *Cutleria*-shoot itself. To this view, however, there are serious morphological objections. In comparing the development of the two stages, it becomes evident that not only is there no special evidence of alternation which could be included under a theoretical alternation of generations, but that even the polymorphism is less evident than at first sight appears.

It is important to note that this polymorphy originates only in the embryonic history, leading to the formation of the embryos designated the *Foot-Embryo* and the *Protonematoid Embryo* respectively; and it is in this that its importance lies. Thus, as far as present data go, the dorsiventral *Aglaozonia* cannot recreate the erect plant vegetatively, nor can the adult *Cutleria* reproduce from the base of its main axis, clothed with rhizoids to form a secondary holdfast, the dorsiventral basal lobes; although a longitudinal section of the attachment-disc shows that at the extreme base it never gets beyond the simple segmentation of the foot (Fig. 10).

While the protonematoid embryo is clearly on the way to a true *Cutleria*-form, it is the foot-embryo which is aberrant in development, in that it presents an anomalous cessation of terminal growth at an early period; and thus the cause of polymorphy may possibly be sought in the solution of the problem as to what induces this arrest of a free-growing axis: that is to say,—Have we to do with the influence of environment on the germinating spore itself, or does it act upon the parent organism? Now, in comparing the *April* cultures of *Aglaozonia* with the *September* cultures of *Cutleria*, at Plymouth, it is difficult to see what

marked differences of light or food-supply there can be at the two equinoxes. Thus, the two stages of germination were carried out in similar vessels, in the same window, at a laboratory temperature varying from 12°–20°, although the average was nearer 14° for the spring and 18° for autumn, and in similar water, in which, as it was not changed, similar gas-conditions must have obtained. Apparently the only factor which varied was that the *Aglaozonia*-plants had been resting throughout the winter at a temperature of 8°–9°, and were in April reproducing on the spring rise to 12°, while the *Cutleria*-plants were vegetating and reproducing throughout July and August at 18°. But although they continued to discharge oospheres on into the autumn at 14°, and possibly at less than 12° in November, the parthenogenetic oospheres all gave true foot-embryos, without exception. As far as present data go, therefore, it would appear that inherited characters may play a certain part; but it has not yet been established that heredity has attained such a degree of importance in the life-history that any alternation is inevitable, much less sexually beneficial. Summing up these data, it remains shown that:—

1. *Cutleria* oospheres, whether fertilized in the Mediterranean (Falkenberg), presumably or actually parthenogenetic in the autumn in the English Channel, developed a foot-embryo, which resulted in definite *Aglaozonia*-thallus and nothing beyond.
2. *Aglaozonia* zoospores produced a recognizable *Cutleria*-form, presenting all the essential characters of a *Cutleria*-thallus with the exception of the fasciation of the branches (protonematoid embryo = *C. multifida* var. *confervoides* Kuckuck),—and under adverse conditions (since the plants died) producing antheridia in great numbers; but also true *Aglaozonia*-discs.
3. *Cutleria* oospheres, germinated parthenogenetically by Thuret, under conditions not described, gave a true protonematoid embryo, which if it had lived would have undoubtedly given a *Cutleria* plant.

And added to these, that:—

4. Janczewski, at Antibes in 1883, germinated a true foot-embryo from fertilized oospores of *C. adspersa*, and this in subsequent development became a dorsiventral structure, closely comparable with many of the Plymouth embryos (Fig. 16). (A special point of interest attaches to Janczewski's cultures, since in them the arrest of terminal growth which forms the special characteristic of the foot-embryo is *not complete*; the young foot-stages being figured as bearing a terminal filament with an intercalary zone of growth.)

5. Reinke, at Naples in 1876, obtained protonematoid embryos, identical with Thuret's *Cutleria*-embryo, from both *zoospores* and fertilized *oospores* of *Zanardinia collaris*. (These stages, which were the most advanced obtained by Reinke for members of the Cutleriaceae, were also distinguished by presenting no trace of the tissue-fusion necessary to form the true adult thallus. They further differ from the Plymouth cultures very considerably in their rate of growth; a definite protonematoid embryo being only obtained by Reinke in three months, while one month at Plymouth gave the furthest vegetative stage observed. Nor was any trace whatever noticed of the peculiar phenomenon, suggested to be pathological, which Reinke describes and figures under the name of 'secondary spores,' in the germination of *Zanardinia*, *Cutleria*, and *Aglaozonia*).

6. Reinke also found the protonematoid embryo of *Zanardinia* growing in its natural habitat, and, as already indicated, the protonematoid embryo of *C. multifida* has been described as reproducing naturally at Heligoland (Kuckuck).

The Relation of Cutleria to Physical Environment.

1. *Means of Dispersal.*

From evidence derived from floating bottles, it is clear that anything that will float will be carried indefinitely along the lines of currents and prevailing winds.

When it is borne in mind that the spores of *Cutleria* and *Aglaozonia* will germinate freely on the surface and float for at least the first month of their existence, and that the film of germinating spores may be at least equal in area to the plant producing them, it is clear that these plants must have practically unlimited powers of dispersal, and that their presence or absence at any given spot must be solely determined by the conditions of external environment.

2. *Relation to Temperature.*

Of the factors of external environment which influence the growth of marine Algae, temperature, light-intensity, the transparency of the water and velocity of current, the first-mentioned is the one most easily measured; and beyond small daily and local variations on the grand annual curve this is so remarkably constant that it is probable that the sensitiveness of marine vegetation to temperature will be found to lie within far narrower limits than in the case of subaerial vegetation. Thus, although seasonal changes are strongly marked, the extreme annual range which at Naples is 20°, is only 12° at Plymouth, less than 8° at Shetland, and as little as 6° at points along the east coast of Scotland (Isle of May). The maximum temperature is found at the end of August, the minimum in February; the sea thus undergoing a steady and rapid rise in early summer, and a rapid fall in late autumn. The former is accompanied by a great amount of light-supply during the summer solstice, the latter by a great diminution in light-intensity towards

TABLE OF SURFACE-TEMPERATURES (C°).

	Jan.	*Feb.*	*Mar.*	*April*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*		*Literature*
PLYMOUTH . .	7 to 8 to 11	6 to 8	7 to 9	8 to 10	10 to 13	12 to 16	13 to 17	14 to 18	16 to 14	14 to 12	13 to 11	12 to 9	Shore waters in summer approximate higher numbers, the lesser being for open sea.	
Naples. . . .	8–10			15–19		20–25		25 to 27	18–22					Berthold.
		15 13			15–19			24–26			18	17		Berthold. Admiralty Chart.
Adriatic . . .	10–15	12–13	11 14		16 12–17		25	23–26	16–22		10–17·5 12–19		Sea temperatures rather than shore water.	Berthold. Admiralty Chart.
Yarmouth . .	3·4	4	4·8	7·9	10	14	16·2	18·6	15·3	13	8·3	5·1	Monthly averages.	Whitley.

Bell Rock . .	4·8	4·7	4·8	5·9	8·1	10·6	12·4	13·3	12·6	11·2	8·4	6·5	Surface.	Fishery Board for Scotland, 1895.
Abertay . . .	4·7	4·8	4·8	6·4	8·4	10·8	12·8	13·6	12·7	10·7	7·6	6·2	Three fathoms.	Ditto.
Heligoland . .	3·7	2·7	3·1	5·2	8·5	12·5	15·6	16·8	16·1	13·1	9·3	6·2	Monthly averages.	Karsten.

the winter solstice. It is clear that in the case of free assimilating plants the light-intensity must be of supreme importance for food-supply and increase in bulk, and there can be no doubt that it is the abundance of light-supply on the ascending part of the temperature-curve which brings forward so rapidly the summer vegetation of the British seas; but on the other hand, these same two factors in the Bay of Naples appear to lead to the death of the greater number of the same plants, and the optimum vegetative period occurs there on the descending part of the temperature-curve with a diminishing light-supply.

Berthold[1], from his observations at Naples, came to the conclusion that optimum light-intensity and velocity of the current were the main factors, and temperature, although important, subsidiary to these. He bases his view chiefly on the manner in which the winter and spring annuals last on in deeper water or shaded situations; but it is clear that these conditions would also imply a lower degree of temperature by affording a protection from the direct heating effect of the sun's rays; while rapid movements of water, by carrying off the heated surface-layers, would produce a similar result. In the case of a plant which requires light for assimilation, it is difficult to isolate the heating effect from the light-intensity of the sun's rays; and as temperatures are easily recorded, it has been thought worth while to collect a few of the available data for different localities. As *Cutleria* grows in fairly shallow water, i.e. less than five fathoms, the temperatures of the surface-water will be sufficient; but it is to be noted that records of surface-temperatures along the shore vary much more than in the open sea, and those alone would be absolutely reliable which were taken on the spot where the plant was growing: for the influence of the land and winds on shallow water in enclosed areas leads to a source of error of possibly 2° C. from the average at Plymouth, while local variations are still greater in the Mediterranean.

[1] Mittheil. Zool. Stat. Neapel, iii. 1882, p. 293.

The observations made at *Plymouth* suggested that *Aglaozonia* vegetated at an optimum of 10°–12°, but was perennial within the annual range of 6°–18°; the zoospores germinated in the spring at 12°, and the optimum range of temperature for *Cutleria* was from 12°–16°, this being accompanied by the great light-intensity of May and June. Under these conditions the thallus was mature and in full reproduction in four months, of which one month may be included in the protonematoid embryo stage. It died (in the Laboratory) at 20°, this temperature being reached at the end of August in shallow enclosed water, and also on the autumn fall to 13°. The autumn fall to 12°, although accompanied by great diminution in light in October and November, led to renewed growth of the *Aglaozonia* which perennated through the winter, growing slowly at its optimum temperature, but stopping if the light-supply was slight, as in dull weather in November and December. The same conditions were also fatal to the whole of the foot-embryos in all stages, which had not yet thrown out *Aglaozonia* expansions.

Complete data are not available for *Naples*, but the same annual period of maximum and minimum obtains, the range being from 8° in January and March to 27° in July and August. As before noted, temperatures to have more than an approximate value require to be taken where the *Cutleria* is growing, and as Berthold gives temperatures of 15° and 17° for February and December, it would seem that *Cutleria* lives in these waters under similar temperature-conditions to those which obtain in the Channel in summer; i.e. it commences growth on the autumn fall in December and matures in four months, completely disappearing in shallow water in April, as the temperature rises to near 20°. At the same time the *Aglaozonia* is perennial over the summer heat of 27°, or at any rate may exist in deeper and colder water. According to Berthold, a stunted growth of *Cutleria* also occurs in deep water in July and August to a certain extent, the temperature at the depth of growth, forty fathoms, being 14°–17°, thus approximating the English temperature. This growth appears

however not to have been investigated, the researches of Reinke and Falkenberg having been carried out on the winter-plants (cf. p. 109).

At *Antibes* the isothermals for surface-temperature very closely approximate those of Naples, and *Cutleria* has here the same annual period.

North Sea, Yarmouth, Heligoland. With a cold winter, late cold spring, and a rapid summer rise, the North Sea presents a variation of from 0° to 18°; the Heligoland curve averaging a degree lower on its rise than that of the western shore. The fall to zero is exceptional, and the mid-winter average for Heligoland is well over 2°, and possibly higher in sheltered localities. More complete data for the occurrence of *Cutleria* in the North Sea would be of great interest, as from the preceding it would appear that here the high degree of temperature necessary to form the mature plant did not obtain, as a rule, throughout a sufficient length of time; and this may possibly be the explanation of the fact that *Cutleria* has been found at Heligoland, but in recent years has only occurred in the protonematoid form as *C. confervoides*. Similarly, *Cutleria* is found at Yarmouth, but is not known to occur along the east coast until the sea at Orkney feels the influence of the warm Atlantic current, although *Aglaozonia* is often common (e.g. Berwick). It is therefore probable that we here reach the minimum heat-supply for the development of the typical *Cutleria*-thallus, owing to the fact that the optimum degree of temperature does not obtain over a sufficient period in the brightest months.

Similarly, *Kiel* has a still greater range, with a high summer temperature but very low mid-winter average, being below 3° during the winter months, and often below zero. This appears to limit the *Aglaozonia*, and neither *Aglaozonia* nor *Cutleria* occur at Kiel or inside the Baltic.

Orkney and Shetland, owing to the presence of warm Atlantic water, show the smallest amount of annual variation, the characteristic feature being the warm winter; while the summer maximum is only 14°, the winter minimum is 4°–5°.

Orkney has a noticeably milder winter than that of Shetland, the February temperature being 6°. It is fully in agreement with previous statements that *Aglaozonia* should here perennate safely and *Cutleria* vegetate in the summer without reaching any great bulk; and it would appear that Pollexfen found all the Orkney specimens to be of the delicate '*penicillata*' variety, which suggests but small amount of growth beyond the protonematoid condition.

Skagerack and Norwegian Coast. From the extensive researches of Pettersson and Hjort, it is known that the temperature of the Skagerack and West Norwegian shores varies from year to year according to the manner in which the summer-heated waters of the Baltic find an outlet into the Atlantic, giving rise in summer to a superficial Norwegian coast-current (the Baltic current) running close along the shore, and thus forming a strip of water from Christiania to Nordland warmer than that of the North Atlantic summer. The Skagerack average temperatures given by Pettersson, especially the higher ones which represent those of warmer seasons, compare very favourably with those of Yarmouth with the exception of the late spring-rise. Christiania Fjord is the warmest portion of the Norwegian seas, and the best Norwegian stations for *Cutleria* occur in this Fjord. In warm winters the surface-temperature does not fall below 5°, while the summer maximum is 17·2°; these numbers falling well within the suggested temperature-limits for *Cutleria* and *Aglaozonia*. On the other hand, as in the North Sea, it is clear that the critical temperatures are reached in passing up the West Norwegian coast, where *Cutleria* is found sparingly, *Aglaozonia* more commonly, extending as far as Nordland but not to Lofoten. Winter-temperatures along the coast again vary in different years according to the relative strength of the Baltic current, now cold at 2° or less, and the open Atlantic at 6°–7°. In warm winters the surface-temperature may be as high as 6° at Hardanger Fjord and as much as 4° at Vikten. On the other hand, the surface-layers lose heat in contact with the extreme low temperature of the air at

Lofoten, and here the surface-temperature of the open sea falls to 1·5° in February; this being again the Kiel average for the same month.

There is therefore a certain amount of evidence in favour of the view that temperature rather than light-intensity is the determining factor as far as the actual existence of these plants is concerned, and that while *Cutleria* vegetates at a mean of 16°, with a range of four degrees above and below, *Aglaozonia* prefers a mean of 10°, with a maximum considerably over 20°, and a minimum below 3°: further, that a continuance of this low temperature limits the existence of both these plant-forms, in that it destroys the perennating thallus, both in the Baltic and along the Norwegian coast.

In order to test these data, observations were attempted at Plymouth in January, 1898, on perennating plants of *Aglaozonia*, both the adult thallus and also the young perennating plants of the first winter germinated the previous summer, large numbers of which were now from ·5 to 2 mm. long, and had been growing for months at an average temperature of 14°.

It is clear that in this case the action of a constant degree of temperature over a longer period of time than was available would be preferable, and the observations were not so successful as might have been wished owing to the difficulty of maintaining a constant temperature over a long period and at the same time maintaining general health-conditions by frequent change of water; and it is probable that the data derived from actual distribution are more likely to be correct than those derived from cultures in the laboratory so long as the difficulty of accurately imitating the natural environment remains. Thus, at 25°–26°, the summer maximum for Naples, young perennating forms remained healthy for ten days, and although a few died, many were alive and well after sixteen days. At 27°–29°, similarly, both young and old plants remained healthy after six days, and there seemed reason to believe that at temperatures below 30° *Aglaozonia* might perennate successfully.

At temperatures above 30°, on the other hand (30°–32°), death occurred sooner or later; young plants dying in 2–4 days; older ones in 4–6 days, dying irregularly in patches. This is of interest as showing the unlikelihood of *Cutleria* crossing the Tropics where the maximum surface-temperature is above 30°.

Experiments at low temperatures were not conclusive, young plants remaining perfectly healthy after being surrounded by melting ice for six days.

Theory of Sexuality.

The theory of the sexuality of the Phaeosporeae, which in point of fact still remains based on the classical researches of Reinke and Falkenberg on *Cutleria*, and those of Berthold on *Ectocarpus siliculosus*, has more recently been called in question by such accurate observers as Kuckuck and Sauvageau [1], who have repeatedly failed in obtaining union of gametes in various species of *Ectocarpus* and allied genera. Thus Kuckuck maintains that *Ectocarpus siliculosus* is constantly parthenogenetic at Kiel, and it may be noted that Reinhardt has observed both copulation and direct germination of gametes in this species at Sevastopol; while Sauvageau in 1895 obtained direct germination in the case of the gametes of seven species of *Ectocarpus* and wholly negative results as regards a sexual process. No one, again, has ever observed sexual fusion in any of the plants of the *Giffordia* section of *Ectocarpus* which possess apparent antheridia, nor again with certainty in any of the Tilopterideae.

The facts in the case of *Cutleria*, however, appear to point to the narrow range of external conditions within which the sexual process can be effected: if these conditions do not obtain, the plant may fall back on parthenogenesis, which in

[1] Cf. Ann. de Sci. Nat. 1896, p. 223.

northern waters is associated with a correlative diminution of the now useless male organs; so that, under extreme conditions, the admittedly asexual mode of reproduction alone remains on the perennating form. There can thus be little doubt that in the case of *Giffordia* and the Tilopteridеae, purely morphological considerations may be a better guide to the theoretical degree of sexual specialization than the physiological observation of the act of fusion of the gametes; and further, that until more complete physical data are forthcoming as to the exact conditions of the experiment, a single positive result must far outweigh many negative ones, and the evidence that the so-called plurilocular sporangia of the Phaeosporeae are not potentially gametangia remains inconclusive.

Nor, on the other hand, do the data for *Cutleria* point so much to an imperfectly differentiated or incipient sexuality, as to an actual and progressive loss of that function; and thus, by analogy, the conception that the primitive *Ectocarpus*-like ancestor of the Phaeosporeae was a sexual plant with isogamous gametes would be strengthened rather than undermined. At any rate it is clear that the actual data for any given plant can only be obtained by actual observations taken at different times of the year at different points of distribution.

Phylogeny of Cutleria.

All generalizations as to the phylogeny of existing Algae must, in the present state of our knowledge, be necessarily more or less founded on the very hypotheses the scientific botanist most desires to prove. At the same time the only proof of such hypotheses at present attainable consists in their complete agreement with ascertained facts; and thus so long as the tentative character of the proceeding is clearly borne in mind, it may become of interest to construct a phylogenetic scheme for the life-history of the genera *Cutleria* and

Zanardinia which will not only include the existing data, but may present some suggestions towards the solution of other algological problems.

The evolutionary specialization of the Cutleriaceae, with which we are here concerned, takes into account the vegetative structure only. A comparison of *Cutleria multifida* (including *Aglaozonia reptans*), *Cutleria adspersa* (and its suggested *Aglaozonia chilosa*), and *Zanardinia collaris* shows that in the structure of the reproductive organs the three types are identical. In both genera the asexual sporangia give rise to a few (6–10) large biciliated zoospores, and thus present an intermediate reduction-specialization as opposed, on the one hand, to the numerous spores from the unilocular sporangium of *Ectocarpus siliculosus*, &c., and to the immotile monospore of the Tilopterideae on the other.

The antheridia show a slight advance on the primitive Ectocarpoid multilocular sporangium, in the more complete delimitation of the antherozoid-tissue, best seen in the skeleton framework remaining after emission of the antherozoids, but they have not attained such a high degree of specialization as that exhibited by the bottle-like antheridium of the Tilopterideae.

In the same manner, a further degree of reproductive concentration has, in correlation with the increase in bulk of the female gametes, reduced the segmentation of a multilocular gametangium to an oogonium of sixteen loculi, each producing a single oosphere; but this again is a lesser degree of reduction than that obtaining in the Tilopterideae with huge solitary oosphere.

Hence, in all three forms of reproductive organ, the Cutleriaceae offer a condition intermediate between the isogamous Ectocarpaceae and the completely heterogamous Tilopterideae; and they may, in view of the present vegetative condition of these two groups, be regarded as descended from a filamentous form which had attained the present comparatively high degree of sexual differentiation before passing beyond the branched filamentous condition in its vegetative structure.

Again, in the Cutleriaceae, the presence of the filamentous stage is clearly marked :—

(1) The mature plant itself is but a fasciated structure of which the growing regions are still in the purely filamentous form ; the assumption of growth by intercalary division, as opposed to the primary apical growth, being general throughout the whole group of the Phaeosporeae. The reproductive portions of the thallus are still wholly in the filamentous condition, and filaments bearing reproductive organs can be induced to grow and attach by rhizoids ; while the attachment-disc is also a mere felted mass of rhizoids ; the only portion of the thallus, in fact, which is not filamentous being the highly specialized assimilative region.

(2) The filamentous condition is characteristic of the embryogeny for a short period in the foot-embryo, but persisting to the adult condition in the protonematoid embryos which produced antheridia in the Plymouth cultures, as also in the female form *C. confervoides* found at Heligoland by Kuckuck.

In the evolution of the vegetative thallus from such a simple filamentous form, in which intercalary growth of the branches supersedes the original apical development, the first step in advance is marked by the *regular segmentation* of the cells of the main axes, by successive divisions by walls in planes at right angles to one another, leading to the more or less regular formation of a multicellular condition such as exists in many Phaeosporeae, e. g. simpler Sphacelarias, *Myriotrichia*, *Desmotrichum*. This massive type of thallus with purely radial symmetry is represented (1) by the foot-embryo, (2) by the basal region of the plant in the protonematoid embryo. In the same way this method of segmentation is ontogenetically repeated in the formation of sterile hair-like branches on the adult thallus.

As an example of such a plant-form in which the cortical cells send out basal lobes forming a dorsiventral disc around the point of attachment of the plant, *Sphacelaria cirrhosa* may be instanced ; and it is clear that in the Sphacelariaceae differentiation has proceeded in two lines from such a simple

form, giving (1) a further specialization of the shoot-system in *Stypocaulon* and *Cladostephus*; (2) a suppression of the shoot system, and reduction to the creeping dorsiventral disc alone in *Battersia*. Such a reduction to a dorsiventral creeping thallus is again in Algae, from a vegetative point of view, a distinctly down-grade specialization. The plant, by adopting a prostrate habit, exposes far less assimilatory surface to the action of light and free-flowing currents which bring both food and oxygen supply; on the other hand, it gains in the struggle to resist the tensions and tractions of wave motion, and will thus exist safely, not only throughout more stormy seasons, but farther up towards the tide-mark.

The relation of *Cutleria* to *Aglaozonia*, from a vegetative point of view, is simply that the two growth-forms representing the extreme cases of specialization of such types as *Cladostephus* and *Battersia* are here combined in a single species, and become fixed, one way or the other, at a very early stage. Thus it is interesting to note that the delicate *Cutleria*-thallus is confined to comparatively quiet waters and depths at least two fathoms below low-tide mark, and possesses a very slender point of attachment in relation to the bulk of the full-grown thallus; while *Aglaozonia* rises, from equal depths, to rocks even above the tide-mark in many localities (Sidmouth). Further, accompanying feeble powers of growth and nutrition, and possibly correlated with them, *Aglaozonia* possesses an increased power of withstanding extremes of temperature.

Beyond the 'massive' stage, the Cutleriaceae make one more advance, which forms the unique characteristic of the order. This consists in the fusions which take place between the axes produced from the independent filaments of the apex of the thallus, leading to a fasciated growth which further presents the complication of dorsiventrality. That this is not only the essential feature of the Cutleriacean type, but is the last and most recently acquired and therefore most mobile character, is suggested by the following considerations.

(1) The specific characters are essentially based on the

characters of the fasciated shoot; variations in this point being still considerable, giving rise to growth-forms which have been regarded as varieties; those with a lesser degree of fasciation being extremely common, especially in localities where external conditions are unfavourable. (Orkney; cp. *C. penicillata*, Lamour; *C. penicillata*, Kützing).

(2) It is now the only character left which has not been observed in cultures, and it would therefore appear to demand the most delicate adjustment of external assimilative conditions.

Considering the three types from the standard of attainment of this assimilative growth-form, it would appear that *C. multifida*, which covers the widest range of temperature in distribution, presents these specializations in a high degree in its sexual shoot, but maintains existence under conditions unfavourable to its development by reduction to a degenerate creeping *Battersia*-like form, which alone persists at the extreme northern limit of distribution.

C. adspersa, with a considerably narrower range of distribution, exhibits a thallus, meagre by comparison with *C. multifida*, but more dorsiventral. Its *Aglaozonia*-stage, suggested for *A. chilosa* by Falkenberg, has not been more definitely isolated; but Janczewski showed that the foot-embryo passed directly into a dorsiventral disc on the approach of summer at Antibes.

Finally, in *Zanardinia collaris* the fasciated shoot, with extreme dorsiventral development, becomes itself prostrate, and by vegetating in the manner of an *Aglaozonia*-disc in the hot summer does away with the necessity for such a basal formation from the embryo; and thus, being itself homotypic and obtaining the perennating advantages of the procumbent growth-form, presents the paradox of becoming degenerate by carrying to extremes the last variation of the family.

During a portion of the time in which these observations have been made, the writer has occupied the Oxford University Table at the Laboratory of the Marine Biological Association at Plymouth; and in acknowledging the goodwill and unfailing

courtesy with which the resources of the Station have always been placed at his disposal by the Director, Mr. E. J. Allen, he would wish to draw the attention of English algologists to the facilities afforded by the geographical position of the Plymouth Laboratory for the study of our native Algae.

Grateful acknowledgments are also due to Mr. E. A. Batters for kind assistance on many out-of-the-way points not easily obtained from the literature.

Postscript.—Since writing the above, Dr. P. Mayer kindly informs me that sea-temperatures taken on different days at Naples during the months December, 1897, and January, 1898, for localities in which *Cutleria* is known to occur, ranged between 13·5° and 13·9°. This adds confirmation, therefore, to the data obtained at Plymouth, which tended to show that the young *Cutleria*-thallus vegetates normally between 12°–14°.

EXPLANATION OF FIGURES IN PLATES VII, VIII, AND IX.

Illustrating Mr. Church's paper on *Cutleria multifida*.

PLATE VII.

All figures drawn with Zeiss D. Oc. 3, and slightly reduced in reproduction.

Fig. 1. Germination of zoospores of *Aglaozonia* (2–3 days).
Fig. 2. Older stage (one week old).
Fig. 3. Protonematoid *Cutleria* producing antheridia (August).
Fig. 4. *Aglaozonia*-disc produced at the base of protonematoid *Cutleria* (August).
Fig. 5. Germination of zoospores of *Aglaozonia* (two weeks old).

PLATE VIII.

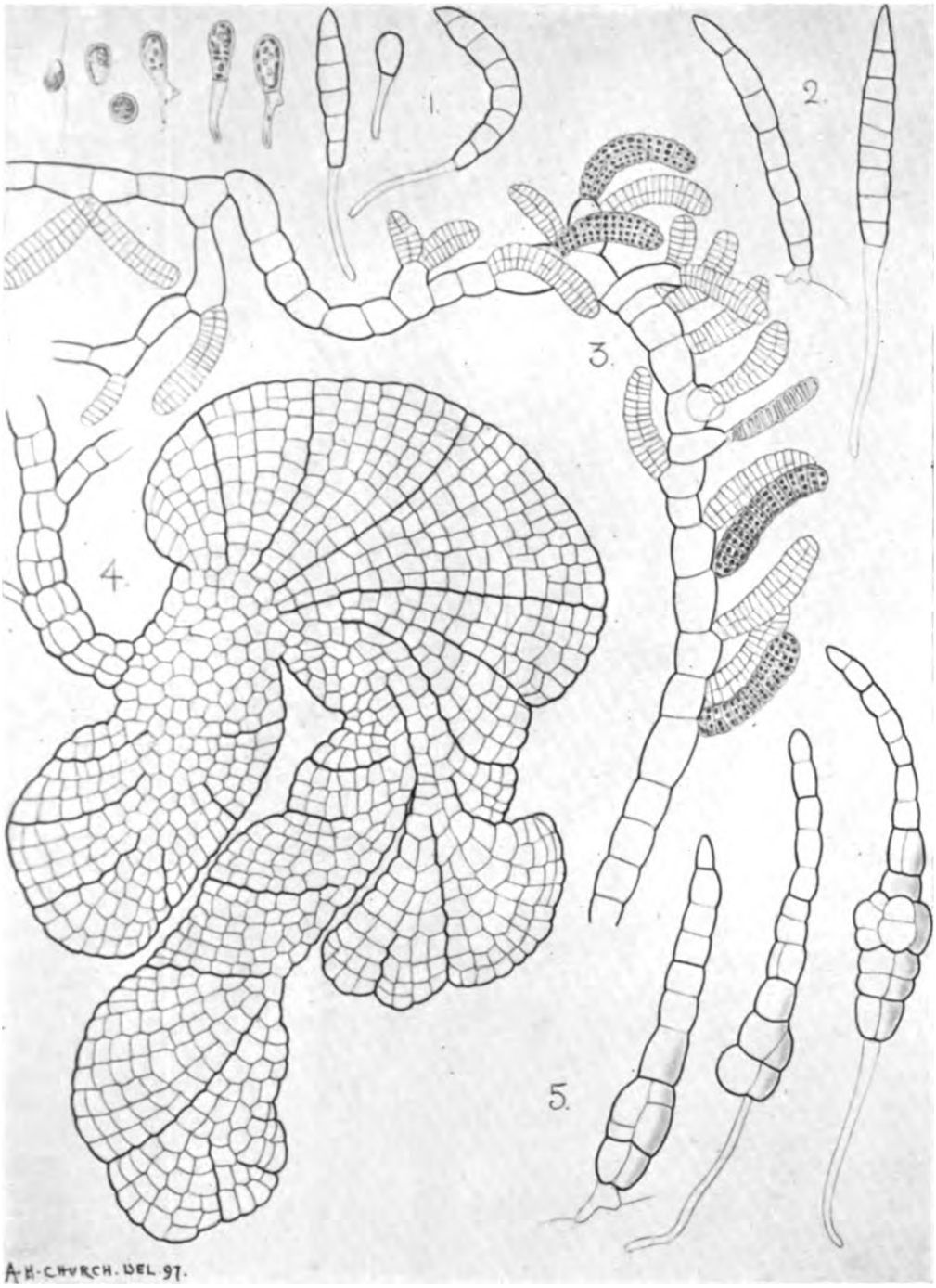
All figures drawn with Zeiss D. Oc. 3, and slightly reduced.

Figs. 6–9. Protonematoid *Cutleria*, three weeks old.
Fig. 9. Embryo with multicellular 'foot'-like base.
Fig. 10. Protonematoid *Cutleria*, four weeks old, with attachment disc.
Fig. 11. Parthenogenetic germination of oospheres of *Cutleria*.
Fig. 12. Embryos, one week old.
Fig. 13. Embryos, becoming multicellular, two weeks old.
Fig. 14. Foot-embryos, three weeks old.

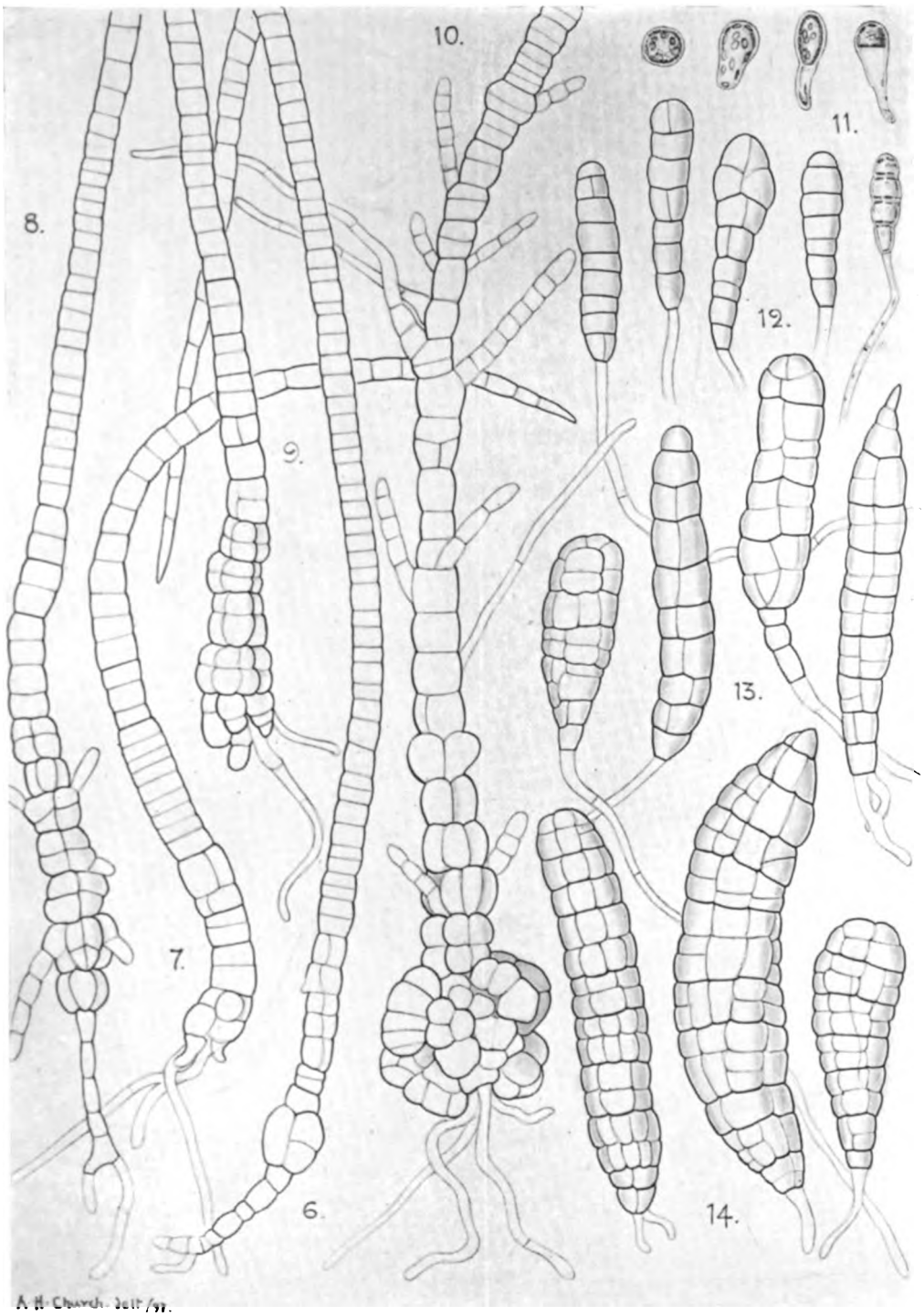
PLATE IX.

Figs. 15–21, Zeiss D. Oc. 5; Figs. 22, 23, Zeiss D. Oc. 1; and all slightly reduced.

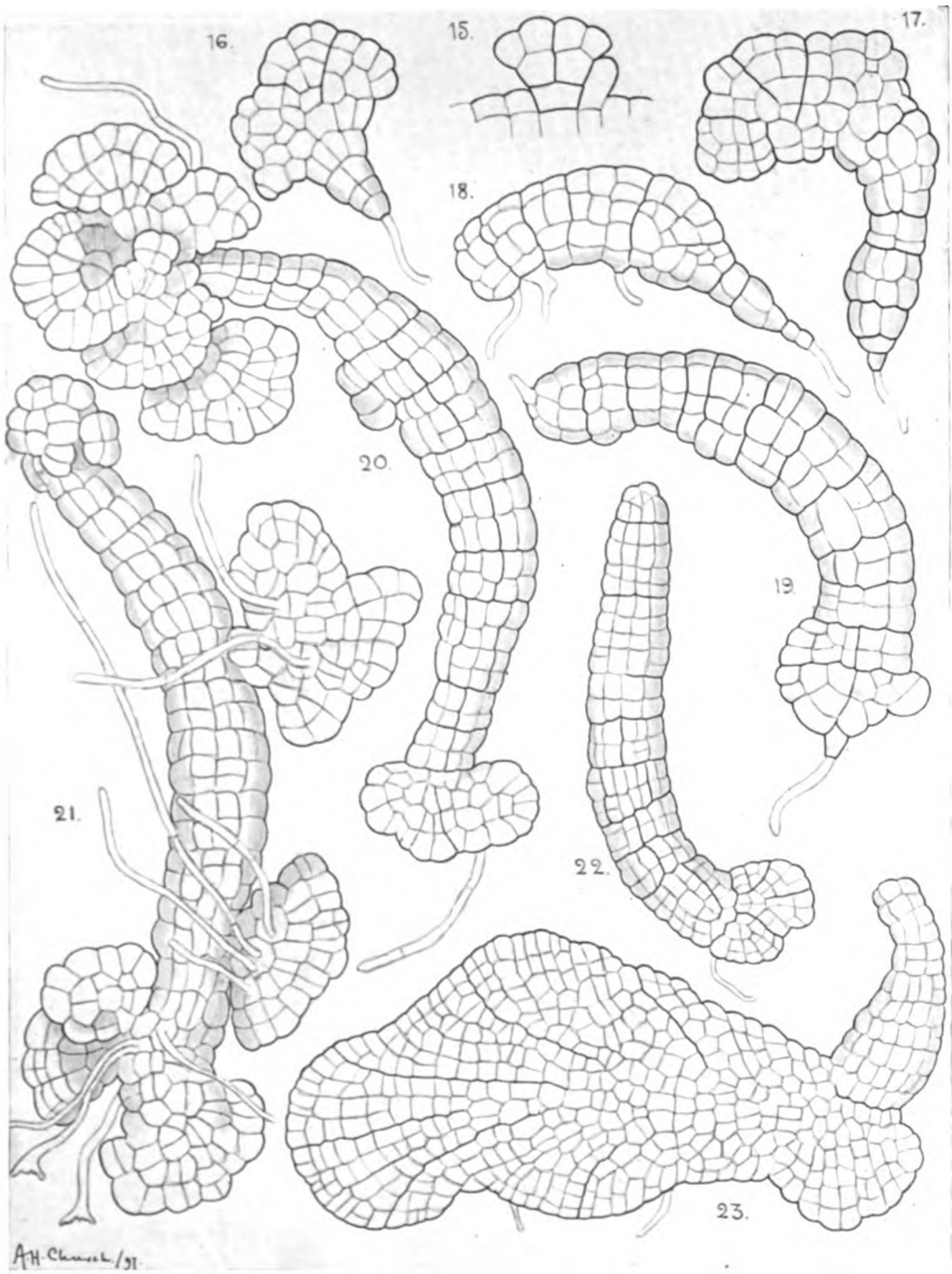
Fig. 15. Development of *Aglaozonia*-disc from a single superficial cell by successive T-shaped walls.
Fig. 16. Small foot-embryo, wholly growing into *Aglaozonia* form.
Fig. 17. Terminal development of disc.
Fig. 18. Lateral development of disc.
Fig. 19. General case of basal development of *Aglaozonia* expansion.
Figs. 20, 21. Three to four weeks old embryos, giving disc-growths at various points. 20, dorsal; 21, ventral surface.
Fig. 22. Older foot-embryo with basal disc.
Fig. 23. Older embryo with *Aglaozonia* expansion well developed (Nov.).



CHURCH.—CUTLERIA.



CHURCH.– CUTLERIA.



CHURCH.—CUTLERIA.

On the Structure of an Ancient Paper[1].

BY

M. DAWSON, B.Sc.

Botanical Laboratory, Cambridge.

IN November last, Professor Marshall Ward handed to me a specimen of ancient paper, with the request that I should attempt to determine the botanical nature of the materials of which it was made.

Examined macroscopically, the paper may be described as a light-brown felt-like substance, consisting of layers—easily separable one from the other—of closely interwoven fibres.

[1] The paper here referred to was one of four pieces of ancient MSS. which were sent to me by Mrs. Gibson of Castle Brae, Cambridge, for examination as to the materials of which they were composed. These MSS. are parts of a series discovered by Mrs. Gibson and Mrs. Lewis at Cairo in 1897, and which proved of some historical interest. They almost certainly came from the Genizeh or lumber-room of the Synagogue in Old Cairo, whose contents Mr. Schechter has brought to Cambridge. The writing on them is Hebrew, and refers to legal matters. Mrs. Gibson informs me that on one fragment there is conclusive evidence of the date, 1038, and further inquiry leads to the conviction that this is one of the oldest fragments of such writing in England. I subsequently received by Mr. Schechter's kindness another fragment of a similar paper. On testing these five papers I found them to be made of flax or some similar fibre, and the subject seemed so interesting that I asked Miss Dawson to go more fully into the matter, which she has successfully done.—H. MARSHALL WARD.

[Annals of Botany, Vol. XII. No. XLV. March, 1898.]

As is well known, many ancient writings are on vellum or papyrus: this, however, was evidently neither a prepared animal skin nor so complicated a tissue as that of the *Cyperus Papyrus*. It clearly was composed of fibres which had by some process been extracted from the tissues of some plant.

After teasing in water and examining with the microscope, the paper was seen to consist of fibres, often occurring in strands, accompanied by elements showing spiral or simple-pitted markings, and here and there by cells of wood-parenchyma or medullary rays. The layers of the paper appear to have been stuck, not woven together; an examination of the dust, shattered from the paper, showed, after treatment with iodine, yellow and bluish-black particles—the latter sometimes lying along the fibres, and giving to them a blue colour. This suggested that some form of starch may have been used in the manufacture, but all attempts to determine its nature were unsuccessful. This alteration of the starch-grains, which had evidently taken place, was probably due to the action of numerous Fungus-hyphae, and groups of Bacteria-like bodies, which could be seen amongst the fibres.

The individual fibres are somewhat cylindrical in shape, with pointed ends, and very narrow lumen. Their walls are thick, and show regular longitudinal striations, but no visible pits. Their breadth is practically constant (·015 mm.), but the length varies considerably: the extreme measurements taken were 6·4 mm. and 3·1 mm.; an average of ten measurements gave 4·25 mm. As, however, so many of the fibres were broken, accurate measurements of length were not possible, and these figures cannot be relied upon. It can, nevertheless, be affirmed that the fibres are shorter and narrower than those of cotton, and are not, like them, flattened and twisted.

They show a marked tendency to tear into fibrillae, often in spirals, and show traces of swellings or kinks. With Schulze's solution, they give a purple colour, with iodine pink, and with iodine and sulphuric acid blue: but no trace of

lignin-reaction can be obtained either with phloroglucin and HCl or with aniline sulphate.

When treated with Cu. Amm., the fibres behave in a characteristic manner: the walls swell greatly—causing the striations to become beautifully clear—and the lumen persists for some time as a very narrow, dark, wavy line, running down the middle. The above results lead to the conclusion that they are some sort of non-lignified bast-fibres.

Among the fibres which seemed likely to be met with as ancient paper-materials are Flax, Hemp, *Boehmeria*, *Broussonetia*, Cotton, and Nettle[1]. We may exclude, by the reactions, such lignified fibres as jute, straw, and wood, met with in modern papers: and, probably, certain Indian and out-of-the-way fibres, as Rice, Bamboo, Daphne, New Zealand Flax, &c., may also be neglected.

A careful examination of the above list gave the following results[2]:—

1. *Cotton* fibres are flat and often twisted; they are broader, longer, and show a wider lumen than those under investigation; and in addition, when treated with Cu. Amm. they coil into spirals before complete solution.

2. *Nettle* (*Urtica dioica*) fibres, like those of cotton, are flat, and somewhat twisted; they are even broader than those of Cotton, and show as wide a lumen. Their walls have distinct longitudinal and transverse striations, and under the action of Cu. Amm. they become swollen and much crumpled, leaving the broad lumen very clearly visible.

3. *Boehmeria nivea* fibres resemble the fibres of the paper in their breadth, narrow lumen, and non-lignified walls, but are clearly distinguished from them by their club-shaped ends, and their characteristic behaviour with Cu. Amm., viz. rapidly coiling into loose spirals.

[1] J. Wiesner, Die Rohstoffe des Pflanzenreiches, p. 447.

[2] See also Cross and Bevan, A Text-book of Paper-making, pp. 30–61.

4. *Hemp* (*Cannabis sativa*) fibres are long and narrow, with a very narrow lumen. Their ends, however, are slightly flattened, and their micro-chemical reactions distinguish them from the fibres of which the paper is made: thus they give a yellowish colour with aniline sulphate, green with iodine and $H_2 So_4$, and in Cu. Amm. they swell greatly, showing longitudinal striations, with a comparatively broad central wavy line.

5. *Flax* (*Linum perenne*) fibres are long and narrow, the walls are thick and non-lignified, and show characteristic swellings or kinks, where fibres have crossed one another. Their behaviour, under the action of Cu. Amm., is strictly comparable to that of the fibres of the paper. The chemical reaction of the walls also agrees closely in the two cases.

A comparison of the above results shows at once that we must exclude the fibres of Cotton, Nettle, *Boehmeria*, and Hemp. I was unable to examine those of *Broussonetia*, but Wiesner's [1] description of them puts them also on one side.

On the contrary, the shape, narrow lumen, and pointed ends, the chemical properties of the walls, their reaction with cupric ammonia, longitudinal striations, tendency to fray into fibrillae, and the traces of slight swellings or kinks, all point to the conclusion that we are dealing with bast-fibres of *Flax*

Considering the subject from a historical standpoint, we learn from De Candolle [2] that Flax was familiar to the Chaldeans, Egyptians, and Hebrews. The plant figures in Egyptian drawings, and, as the microscope has revealed, the bandages, used as mummy-wrappings, were made of linen. Moreover, the annual Flax was cultivated for thousands of years in Mesopotamia, Assyria, and Egypt, and it was, and still is, found wild in the districts lying between the Persian Gulf and the Caspian and Black Seas.

[1] J. Wiesner, Die Rohstoffe des Pflanzenreiches, p. 459.
[2] De Candolle, Origin of Cultivated Plants, 1884, pp. 123–130.

It is interesting to note also, that Karabacek, in his preface to the descriptive catalogue of the Archduke Rainer's Collection of MSS.[1], gives reasons for concluding that the Arabs learnt the art of paper-making from plant-fibres, from the Chinese, about A.D. 751.

We are not, therefore, guilty of any anachronism in assuming that, by the year 1038—the date which has been assigned to this paper—the process of manufacturing paper from the fibres of the Flax-plant was both known and employed.

[1] Führer durch die Ausstellung Papyrus des Herzog Rainer, 1892, Th. I, pp. xvii-xxiv.

NOTES.

CORRELATION OF GROWTH UNDER THE INFLUENCE OF INJURIES.—In the paper on this subject which appeared in the Annals of Botany, Vol. xi, No. XLIV, December, 1897, reference was made on p. 513 to Laurent's valuable paper, Études sur la Turgescence chez le Phycomyces, but by an oversight the name of the author was omitted.

C. O. TOWNSEND.

GELATINE AS A FIXATIVE.—Microtome-sections passing through embryonic and parenchymatous tissues embedded in paraffin are sufficiently fixed to the microscope-slide, for staining purposes, by their own simple adhesion to the glass. This, however, is not the case when the section comprises a large proportion of woody tissue. For such preparations, collodion, agar-agar, and albumen have been recommended as fixatives. The first of these, so far as my experience extends, is the most certain. But it has the disadvantage that with its use the paraffin-section cannot be floated out on water on the slip and caused to flatten out by gentle warmth. The same objection applies to albumen [1], and in addition, I have found it to be very easily coloured by stains (especially the blue dyes) which are often essential to use in microscopic work. I have no experience with agar-agar, but Zimmermann states that it becomes dyed with haematoxylin—one of the most important stains, and that the sections often come loose from the glass during the staining and washing manipulations. This latter objection, perhaps the most vexatious of all, applies, to some extent, to albumen also.

Recently I have used as a fixative a dilute solution of gelatine in a watery solution of bichromate of potash. The solution should be quite fluid at 10° C. In use the ribbon of paraffin-sections is laid on a drop of this solution on the slide. Wrinkles in the sections may

[1] I used the preparation given by Zimmermann, Bot. Mikrotech.

be removed by gently warming the slide over a flame. Then the superfluous fluid is drawn off by blotting paper, and the gelatine is allowed to dry and harden. During this process it should be exposed to a bright light. The action of the light on the bichromated gelatine renders it quite insoluble even in warm water, and so removes all danger of the sections becoming detached from the slide. The bichromate of potash in the gelatine has this additional advantage, that after exposure to light it prevents the latter from taking up the dyes used as stains. So far as I have at present tested it, this preparation of gelatine is unaffected by saffranin, fuchsin, acid fuchsin, haematoxylin, iodine green, gentian violet, and aniline blue. With aniline blue, however, a precipitate is sometimes formed along the line which formed the edge of the paraffin ribbon and in cracks in the ribbon; but in no case is the substance of the gelatine itself stained, and so it offers a marked advantage over albumen and agar-agar.

HENRY H. DIXON.

TRINITY COLLEGE, DUBLIN.

LATHRAEA SQUAMARIA.—I find that in my paper on this subject, which appeared in Annals of Botany, Vol. xi, 1897, p. 385, I omitted to include in my references to the literature the observations of C. Darwin and F. Darwin, of which an account is given in 'The Power of Movement in Plants' (footnote, p. 85). The authors show that *Lathraea* can excrete large quantities of water underground, and state that the water is secreted by glands lining the cavities of the scales.

PERCY GROOM.

On the Development of the Leaf and Sporocarp in Marsilia quadrifolia[1], L.

BY

DUNCAN S. JOHNSON.

With Plates X, XI, and XII.

ALTHOUGH *Marsilia* and the related *Pilularia* have been frequently studied during the present century, the exact origin and morphological significance of the sporocarp has never been satisfactorily made out in either genus, the chief reasons for this being apparently the complexity of the apical bud and the dense covering of trichomes over all the younger parts. The present work was undertaken at the suggestion of Dr. J. P. Lotsy, then of the Johns Hopkins University, in the hope that a detailed study of the development of the leaf and the sporocarp of *Marsilia* would give some indication of the morphological nature of the latter. The work has been carried on during the winters '95–'96, '96–'97, in the biological laboratory of the Johns Hopkins

[1] Accepted as a thesis for the Degree of Doctor of Philosophy by the Board of University Studies of the Johns Hopkins University, Baltimore, U.S.A., June, 1897.

[Annals of Botany, Vol. XII. No. XLVI. June, 1898.]

University, under the stimulating direction of the late Prof. J. E. Humphrey, to which any value the work may have must be largely attributed.

The material was collected, partly at New Haven, Conn., through the kindness of Prof. W. A. Setchell, and partly at Cromwell, Conn. It was fixed in 95% alcohol, 1% chromic acid or a 5% sublimate-acetic mixture. For staining, gentian-violet or Mayer's haemalum, the latter either alone or in combination with Bismarck brown, were found most satisfactory.

The Development of the Leaf.

Our knowledge of the development of the leaf in *Marsilia* is due almost entirely to the work of J. Hanstein on the embryo, and frequent reference to this will be necessary in the following pages.

The leaves arise in two rows, one on each side of the median line on the dorsal surface of the stem. Each leaf is developed from a typical two-sided apical cell formed from part of one of the dorso-lateral segments of the tetrahedral apical cell of the stem. This apical cell of the leaf is recognizable when the stem-segment in which it is formed is only the third or fourth in its series from the apical cell of the stem. It is larger in size and projects more than the neighbouring cells (*L*, Fig. 1), and its position is such that its edges are directed toward the base and the apex of the stem.

Hanstein ('65) has already shown the shape and position of the apical cell to be as above described in all but the very earliest leaves of the embryo of *Marsilia*, which agrees thus with most other Leptosporangiates that have been studied. They have been thus described by Hofmeister ('62) in *Aspidium*, by Kny ('75) in *Ceratopteris*, by Klein ('87) in *Polypodium*, by Meunier ('87) and Bower ('89) in *Pilularia*, by Campbell ('87) in *Onoclea*, and by Bower ('89) in *Trichomanes*. *Pteris* is apparently the only known case where,

according to Hofmeister ('62) and Klein ('84), the position of the two-sided apical cell is transverse to the stem.

This apical cell of the leaf in *Marsilia* continues its growth and activity, cutting off segments alternately toward the right and left of the young leaf, which has its ventral side facing toward the stem-apex. When about fifteen or sixteen pairs of segments have been formed, the activity of the apical cell is ended, probably by a periclinal wall like that seen by Sadebeck ('73), Kny ('75), and Bower ('84) in other Lepto-sporangiates, and that to be described later in the sporocarp of *Marsilia*. The great regularity of the segments of the leaf in *Marsilia*, as well as the fact that certain of the cells remain of the full length of the segment (Fig. 3), make it possible to determine quite definitely the number of segments formed (Fig. 2). The only doubt is in regard to the first segments, some of which fuse with the tissue of the stem, and it is thus not certain that the segment numbered 1 in Fig. 2 is not really the second one. The young leaf is about 1 mm. long and ·15 mm. in diameter at the base when the activity of the apical cell ceases, and is a slightly tapering conical organ curved upward (Fig. 2) and ventrally over the stem-apex. It is almost exactly circular in cross-section until the formation of the pinnae begins, and is not at all spatulate as described by Hanstein ('65) in *M. Drummondii*, and by Campbell ('96).

The segments of the apical cell of the leaf, or 'primary marginal cells' of Hanstein ('65) and Sadebeck ('74), are nearly semicircular blocks with the upper and lower surfaces slightly concave toward the apex (Figs. 4–6). The first division-wall appearing in these segments is a longitudinal and radial anticline (*I*, Fig. 6), cutting off about one-third of the segment toward the dorsal side to form what we may call a *section*, and leaving on the ventral side a secondary marginal cell. Wall I is apparently the 'tangential wall' of Sadebeck ('74), and *section* I is the 'Schichtzelle' of Hanstein ('65), but this terminology does not seem appropriate when the real position of this and later section-walls is taken into

account, since it refers to the position of these walls at the intersection with the surface, when the thing of importance is, as we shall see, their position in the interior.

The second wall formed in the segment is also a longitudinal anticline in the secondary marginal cell, and nearly parallel to the inner or median border of the segment (*II*, Figs. 4, 6); and thus is formed a tertiary marginal cell (*mc.*^{3}, Fig. 6). This latter is then divided by a transverse anticline (*t. a.*^{1}, Figs. 4, 5), the radial wall of Sadebeck, into an upper and a lower tertiary marginal cell. Then in each of these further section-walls, to the number of three, are formed near and parallel to the dorsal and ventral sides alternately (*III*, *IV*, *V*, Figs. 4, 5, 7, 8), and there are thus formed two marginal cells of the sixth grade in each segment (*mc.*^{6}, Fig. 8). In a less frequent type of division only four section-walls are formed, and the ultimate marginal cells are thus of the fifth grade.

The Petiole.

The first nine or ten pairs of segments of the leaf go to form the petiole, and the six primary divisions of the segments (taking the type where the ultimate marginal cell is of the sixth grade) break up into cells, as will now be described. About the same time that wall II is formed, there appears in section I a pericline (*pl. w.*, Fig. 6), cutting off at the inner end a part of the plerome contributed by this section to the longitudinal bundle of the petiole. This is followed by a longitudinal and radial anticline, the *halving anticline*, cutting the outer cell into two (*h. a.*, Figs. 5, 7, 8). Each of the other sections and the marginal cell in turn cuts off plerome at the inner end (*pl. w.*, Figs. 7, 8), but no halving anticline is formed in any of them. Then there appears a pericline in the outer end of the halves of section I, in the outer ends of each of the other sections and of the marginal cell, separating a layer of outer cells which give rise to the epidermal structures of the petiole from an inner one of cells forming the mesophyll. We may for the sake of brevity,

though not with strict propriety, call these layers dermatogen and periblem, and the pericline itself a dermatogen-wall (*d. w.*, Fig. 7, *d.*, *pb.*, Fig. 22).

The dermatogen soon splits by another pericline into hypodermal and epidermal layers (Figs. 8–10, 22), each of which remains of one cell in thickness even at maturity, though numerous anticlines, both longitudinal and transverse, are formed in each. On the line of the median wall, of each section-wall and of the halving anticline of section I, there are formed intercellular spaces (*a.*, *c.*, Figs. 8, 22) between the periblem and hypodermis, which are the beginnings of the fourteen (primary) longitudinal air-canals of the petiole (*a. c.*, Fig. 9).

The single periblem-cell of each half of section I cuts off by a pericline at the inner end a second portion of plerome (*pl.*, Figs. 8, 22). Then each of the remaining periblem-cells of section I, the single periblem-cell of each of the other sections and that of the marginal cell, divides by a pericline into an inner and outer cell (Figs. 8, 22). Of these the inner cell divides by anticlines and periclines to form the loose mesophyll-tissue of the mature petiole (*mp.*, Figs. 8–10), while the outer cell gives rise to both the longitudinal and transverse partitions between the adjacent air-canals (*p. c.*, Fig. 8). These latter cells swell in the middle (as seen in cross-section of the petiole) and grow out at the ends into papilla-like tips (Figs. 8, 11, 12), touching their fellows of the adjacent sections, but leaving an intercellular space surrounding each tip. The tips thus formed are soon cut off by longitudinal anticlines (Figs. 8, 11), forming a pair of nearly isodimentional cells (*c. p. c.*, Figs. 8, 11) in each air-canal, opposite each primary partition-cell, of which there are usually eight in the length of each segment. From these eight pair of cells are developed the eight transverse partitions of the air-canal in each segment. These remain one cell thick even at maturity, but during their later development many intercellular openings or pores are formed, allowing the passage of air through them (*c. p. p.*, Figs. 9, 10, 13).

The portion remaining of each primary partition-cell (*l. p. c.*, Fig. 11) grows in a radial direction and splits by periclines, while it at the same time grows in the direction of the length of the petiole and divides by transverse anticlines. Thus are formed the longitudinal partitions between the adjacent air-canals, which are also one cell in thickness (Figs. 9, 10, 13). As each of these primary longitudinal partitions elongates with the lengthening of the petiole, it is seen (*l. p. c.*, Fig. 12) that the primary cross-partition cell at one end is nearer the upper wall and that at the other is nearer the lower wall. Then when the first transverse anticline is formed it is somewhat oblique and forms thus two wedge-shaped cells, each with a cross-partition cell at the broad end and none at the narrow one (*l. p. c.*, Fig. 12). The cross-partitions in adjacent canals are thus alternate. These wedge-shaped cells continue to elongate and divide by transverse anticlines (Fig. 13) till in the mature petiole the cross-partitions are far apart. Here again in the longitudinal partitions we find at maturity many small intercellular openings or pores, the 'méats' of Meunier (*l. p. p.*, Figs. 10, 14).

When the epidermal surface of a section is two cells broad and four cells long (Fig. 15), there is cut out of each cell, by a semicircular anticline at the upper end, a small cell which gives rise to one of the numerous trichomes that clothe the young leaf. The rest of the epidermal cell then divides further by anticlines (Fig. 15), and more trichomes arise in the cells thus formed, while the epidermal cells at maturity become much elongated (Fig. 16). Each trichome-cell grows out beyond the surface of the epidermis, and swells to a knob at the outer end (*tc.*, Figs. 2, 3, 9), which soon elongates in the direction of the length of the petiole. On the lower or basiscopic side it projects but little (Fig. 3), while it grows out toward the apex of the leaf to the long multicellular hair (*tc.*, Fig. 17) that is supported by the basal or stalk-cell which remains wedged in between the epidermal cells (*b. c. tc.*, Figs. 9, 16, 17); later in the development most or all of these trichomes are cast off, and the petiole thus becomes naked at maturity.

Stomata also occur on the petiole, but apparently not until quite a late stage, and their development was not studied.

When the longitudinal partitions are about three or four cells in width (radially), longitudinal rows of mesophyll-cells, usually one opposite each partition, have become specialized to form the so-called tannin-sacs (*t. s.*, Figs. 9, 10). The cells composing these are, like the surrounding mesophyll-cells, about twice as long as broad at maturity and rounded off laterally, forming many small intercellular spaces connecting with the large air-canals.

While the dermatogen and periblem have been developing as described above, the plerome of sections I–IV has given rise to the axial vascular bundle of the petiole. The plerome of section II divides by two longitudinal anticlines into quarters, of which the one in the angle between I and III never divides further in any direction, but forms the large trachea of its side of the bundle (*tr.*, Figs. 3, 8–10, 22). The nucleus of this cell may divide many times, so that in a trachea of half a millimeter in length (Fig. 3) we may find twenty-five or thirty nuclei, but these and all other protoplasmic contents disappear later and the end-walls assume the characteristic oblique position, always with the dorsal edge directed towards the base of the leaf.

The remaining three quarters of this section and all the plerome of sections I, III, and IV break up by numerous longitudinal walls (Figs. 8–10), and later by fewer transverse walls (Fig. 3), to form the remaining tissues of the bundle which later still develops a bundle-sheath (*b. s.*, Figs. 9, 10). The portion of the marginal cell within what we have called the plerome-wall never forms any part of the vascular bundle, and the same is usually true of the same portion of section V, though it does occasionally form a small portion of it (Fig. 9).

The Lamina.

Just before the activity of the apical cell ceases, the tenth and eleventh (or eleventh and twelfth) segments on each side begin to grow out laterally and ventrally to form the first

pair of pinnae (p^1., Fig. 18). Each pinna is formed from the whole length of one segment (usually the lower one) and most, but not all, of a second (Fig. 19). In this respect the pinnae resemble those of *Ceratopteris* (Kny '75) and differ from those of *Asplenium serpentini* (Sadebeck '73) and *Onoclea* (Campbell '87), in which the pinnae are equal in extent to the segments.

Soon after the apical growth ceases, the segments beyond the first pair of pinnae, except part or all of the one next the lower pinna on each side, begin to swell out in a similar manner to form the terminal pair of leaflets.

In a transverse section of the leaf through the pinnae, which is practically the same for both pairs, we see that the swelling mentioned is due to the continued activity of the marginal cells (Fig. 20). No pericline is formed in these, as in the marginal cells of the petiole, but anticlines parallel alternately to walls IV, V are formed continually until the pinna is two millimeters broad or more (Fig. 21). The additional sections thus formed divide like the earlier ones by periclines to form the three meristem-layers of the lamina. The marginal cells also divide frequently by anticlines perpendicular to the edge of the pinna, thus constantly increasing in number and giving the pinna a fan-like shape with rounded outer edge formed by the actively dividing marginal cells (Fig. 19), as was shown by Hanstein. The pinnae are directed more ventrally than laterally from the petiole, and the upper pair soon come to have their upper or ventral surfaces nearly in contact, while the lower and older pair fold together (Fig. 21) to enclose the younger ones between them in the bud (Fig. 25).

A branch of the axial bundle is given off to each pinna, which branches to form the anastomosing veins characteristic of *Marsilia*; but the exact development of these bundles of the pinna was not studied, and I cannot state whether they arise, as Sadebeck ('74) has shown them to, in *Asplenium*. The epidermal cells of the leaf give rise to stomata on the upper or both sides, and to deciduous trichomes like those of the petiole.

THE SPOROCARP.

The bean-shaped sporocarps of *Marsilia quadrifolia* are usually borne in pairs, the stalks of the two uniting below, as shown by A. Braun ('70), to form a common stalk joining the petiole of the fertile leaf on its inner side near the base. Occasionally but a single sporocarp is found, or two with stalks separately inserted on the petiole, or more rarely three or four, usually with a common stalk. In the half-grown sporocarp we find the smaller or younger one of the pair is borne on the side of the stalk toward the petiole. If sporocarps occur on any leaves of a given branch they are usually found on all.

Plants of *Marsilia* which were left out of water in September by the drying up of a pond, matured many more sporocarps than plants growing where the water-level was constant. Although the latter had an equal number of young sporocarps in July, nothing but small and often shrunken rudiments were found on most of the plants in September; these might, however, be borne on large and well-developed petioles, so that there is no regularity in the retardation in development of the fertile leaves.

Bischoff ('28) says the sporocarp of *Marsilia* arises as a slight prominence on the anterior side of the petiole, while Mettenius ('46) states that it originates endogenously, and later breaks through the epidermis of the petiole to form a solid mass of tissue, in the interior of which later the sori and canals are developed. The youngest sporocarp studied by Russow ('72) had a two-sided apical cell, but was already differentiated into stalk and capsule (probably about the stage of that shown in Fig. 42). He thought the soral canals arose by the splitting apart of certain cells in the interior of the capsule and the formation of pits on the ventral surface into which these slits opened, to close again later by the growth of the cells on the ventral surface. On the walls of these canals arose the 'soral cells,' in each of which later a tetra-

hedral apical cell was formed, which cut off a number of segments that, according to Russow, gave rise to the placenta with its vascular bundle and to the microsporangia, while the apical cell itself finally became the macrosporangium. Goebel ('82) states that the soral canals of *Marsilia* are external in origin, and that the sporangia arise from superficial cells; Büsgen ('92) describes the first rudiment of the sporocarp as 'eine scheinbare grosse Lücke' in the tissue of the young leaf, and he thinks it probable that all the soral cells of each sorus are derived from a single superficial cell of the ventral surface. The placenta, microsporangia and macrosporangia, he states, are formed as Russow has described from these soral cells.

According to my own observations on *M. quadrifolia*, the sporocarp makes its appearance when the young fertile leaf consists of about six or seven pairs of segments, and thus long before the appearance of the lamina. It is developed from an apical cell exactly like that of the leaf, formed in one of the ultimate marginal cells of the inner side of the petiole (*F. m. c.*, Fig. 22) and placed transversely to the latter. The marginal cell involved may be either the upper or lower of (apparently always) the second segment of the inner side of the petiole, though, because of the crowding together of the various rudiments of the bud, this could not be made out with certainty (*F.*, Fig. 23). The sporocarp is thus not, strictly speaking, epidermal in origin, but resembles closely in its origin the single sporangium of *Lygodium* from a marginal cell of the fertile pinnule, as described by Prantl ('81).

The apical cell of the sporocarp thus formed goes on cutting off segments, alternately toward the base and apex of the leaf, or to the right and left of the sporocarp itself, until about twenty-three pairs of segments are formed. It thus gives rise to a papilla, much like the very young leaf, which bends laterally to grow up beside the petiole with its ventral side facing in the same direction (Fig. 24), and then bends ventrally upon itself at the point where the stalk joins the capsule (Fig. 25). Finally, at about the time that the

activity of the apical cell is ended, by the appearance in it of a periclinal wall, the capsule or upper part of the sporocarp lies with its ventral side nearly in contact with that of the stalk (Fig. 31 *a*). The capsule is at this time about 1 mm. long, and the sori at the base about as far developed as that shown in Fig. 36. There is never any curling in of the extreme tip of the capsule, suggesting the circinate coiling of the leaf, and the sharp bending mentioned above is partially straightened out later, as Russow has shown, by the more rapid growth of the capsule at the base.

The shape and size of the segments of the apical cell are very nearly like those formed in the leaf, and their earlier divisions are exactly the same. Walls I and II (Fig. 27) are followed by the transverse anticline dividing the marginal cell (*t. a.*[1], Fig. 34), and wall III is formed in the same position as in the leaf. This is followed, however, by another anticline parallel to III (*IV*, Fig. 27), and then the regular alternation is resumed, wall V being on the ventral side, and VI, the last wall, on the dorsal side of the marginal cell (Fig. 28). We thus have one more section dorsal to the marginal cell than in the leaf, and the ultimate marginal cell is of the seventh grade instead of the sixth.

The position of the section-walls given above is in general that found in all of the segments of the sporocarp, but certain exceptions are worthy of note. Thus wall IV, instead of running through to wall II as usual, often bends down to meet wall III at some distance from II (dotted line, Fig. 27). This type of division, however, was never seen in the lower or basiscopic marginal cells of the soral segments of the capsule. Again, section V is usually narrower in the basiscopic marginal cells of the soral segments of the capsule (*V*, Fig. 34), and hence the basiscopic ultimate marginal cells, which are evidently 'the sorus mother-cells' of Büsgen, are the largest ones of the ventral side of the capsule. Finally, any marginal cell of the sporocarp, except the basiscopic one of the soral segments, may form a pericline instead of wall VI, and thus make the ultimate marginal cell of the sixth grade. This

behaviour is apparently analogous to that of certain marginal cells of the fifth grade in the leaf.

When the second sporocarp of a pair is formed, it arises usually from a marginal cell of the second or third segment of the first sporocarp on the side of the latter toward the petiole on which it is borne (F^2, Fig. 26), and a third probably arises in the same way from the second. The position of the apical cell of this second sporocarp, with reference to the first, is transverse, like that of the apical cell of the first with reference to the petiole. This mode of origin of the younger sporocarp from older ones shows that the common stalk of the pair is simply the portion of the stalk of the older one below the point of origin of the second, and the same is true of the stalk common to the second and third sporocarps of a trio. It is probable that where two or more sporocarps are inserted on the petiole by separate stalks, as happens occasionally in *M. quadrifolia*, and constantly in forms like *M. polycarpa*, we should find them to originate from marginal cells of successive segments of the petiole, but the early stages of this type were never seen.

The Stalk.

The further development of the seven divisions of each segment mentioned above differs in the different regions of the sporocarp. In the four or five oldest pairs of segments that form the stalk, their later history is very like that of the segments of the petiole, except that the axial bundle is here formed entirely from the plerome of sections I and II and a part only of that from section III. All the remaining portions of these segments, and all of the other segments, give rise to mesophyll and to hypodermal and epidermal structures, but no tannin-sacs are formed among the mesophyll-cells, and only very small air-canals between these and the hypodermis. A structure is thus formed of smaller diameter than the petiole, and of much firmer tissue, which swells out at the upper end (*l. t.*, Fig. 44) to form the lower tooth of the capsule.

THE CAPSULE.

In the seventeen or eighteen segments forming this part of the sporocarp, we find that plerome- and dermatogen- walls are formed in each section, as in those of the petiole (Figs. 27, 28), and the halving anticline of section I is followed by periclines cutting off another portion of plerome from each half. The dorsal bundle, which is a continuation of the axial bundle of the stalk, is made up entirely from the plerome of section I (*d. b.*, Fig. 31), some of the cells of which differ from all others of the capsule by remaining of the full length of the segment. The bundle is thus much more restricted in origin than that of the petiole.

The dermatogen-layer in the capsule splits, as in the leaf, into epidermal and hypodermal layers, of which the former remains one cell in thickness and gives rise to stomata and deciduous trichomes, while the latter divides (*hy.*, Figs. 29, 31) to form the two layers of thickened cells of the wall of the mature capsule, differing thus from the hypodermis of the leaf (Fig. 10). The periblem of the capsule gives rise to the several layers of loosely packed cells between the vascular bundle and the hypodermis, and between these cells and the latter are developed the numerous but small air-canals confined mostly to the dorsal side (*a. c.*, Fig. 29) and separated by partitions arising like those in the petiole.

In the first three segments at the base of the capsule no sori are formed, but there is formed in the youngest pair from the plerome of sections III and IV, in a way to be described in treating the soral segments, a forked lateral branch of the dorsal bundle (Fig. 44). The plerome of sections II, V, and VI of this pair of segments, and all but section I of the next older pair, is apparently devoted to the formation of the basal portion of the gelatinous ring on which the sori are borne when the capsule bursts. In the oldest pairs of segments there is formed a two-layered wall of thickened cells, like those of the hypodermis, stretching completely across the

base of the capsule (*b.w.*, Figs. 31 *a*, 42, 44). In the periblem above the dorsal bundle in these segments and in several of the older soral ones is developed a wall of thickened cells, enclosing between it and the dorsal hypodermal wall a lens-shaped mass of looser cells, the 'linsenförmige Raum' of Russow (*l. c.*, Fig. 44). There is an opening into this space just above where the dorsal bundle pierces the basal wall, and another at the anterior end, out of which there projects a rod of brown-walled cells (*br.*, Fig. 44). The epidermal cells above this cavity swell out later to form the upper tooth of the capsule (*u. t.*, Fig. 44).

Each segment of the next eight or nine pairs give rise to a sorus. Section I in these segments develops much as in the segments of the stalk, as we have seen above. But the other sections have a peculiar history. Sections III. IV, and VI, dorsal to the marginal cell, widen rapidly at their outer ends, while sections II and V do not. The ultimate marginal cell is thus pushed around to a ventral position, the interpolated section IV contributing largely to this end (Figs. 27, 29; cf. Figs. 8, 9). Finally, all other cells in this region grow out beyond the cells formed from the basiscopic ultimate marginal cell, and grow together over the outer ends of them, completely enclosing them (Figs. 29–33).

The Vascular Bundle-System.

The dorsal bundle, arising from the plerome of section I in all the segments of the capsule, gives rise in each soral segment to a lateral branch that runs down back of each sorus, between this and the lateral wall of the capsule. At a point about opposite the middle of the sorus the lateral bundle splits to three branches, as Russow has shown. Two of these (*l. b. f.*, Fig. 33) continue on in the course of the single part of the bundle, while the third turns abruptly inward to connect with the placental bundle of the sorus (*p. br.*, Figs. 33, 44). The dorsal and single portion of the lateral bundle arises from the basiscopic half of the plerome

of section III, and from a part of the same region of section IV (*l. b.*, Figs. 29, 31, 32). Of the two outer forks of this bundle one arises in the basiscopic quarter of the acroscopic half of the plerome of the same segment (*l. b. f.*, Figs. 35, 39, 40), and the other from the acroscopic quarter of the acroscopic half of the next older segment. These forks are formed very early, but grow in length with the sorus, and finally on beyond it to the ventral edge of the capsule (Figs. 33–44), where their ends become connected in a more or less regular way with those of their fellows of the same side of the capsule (*l. b. f.*, Fig. 44). The third or placental branch of the lateral bundle arises from a part of the plerome of the basiscopic half of section VI (*pa.*, *br.*, Figs. 31, 32, 33, 42), and the placental bundle with which this connects is developed from the plerome of the same part of this section (*pa.*, *b.*, Figs. 30, 32, 36, 41, 42).

The Sori.

Of the sections on the ventral side of the marginal cell, the plerome of section II develops ultimately into the large-celled tissue of the dorsal portion of the gelatinous ring (*pl.*^{2}, Figs. 29, 31, *g. r.*, Figs. 32, 33), described by Hanstein ('62) and Russow. The plerome of section V grows around under the inner end of the marginal cell (Figs. 28–32), and probably takes part ultimately in the formation either of the gelatinous ring or perhaps of the stalks by which the indusia are attached to the latter, but this was not determined with certainty. The periblem of both these sections apparently develops very slightly, and seems to form a part of the stalk of the indusium (*pb.*, Figs. 28–31), but the boundary between this and the dermatogen soon becomes indistinguishable. The dermatogen of both sections grows rapidly in a radial direction (*d.*, Figs. 28–31, *o. ind.*, Figs. 32, 33), and gives rise to that portion of the indusium on the median side of the sorus. The outer or ventral cells of these sections soon grow over laterally to meet section VI, and thus enclose the cells of the sorus, while certain cells of these just below the ventral wall give

rise to part, if not all of the ventral portion of the gelatinous ring. The inner portion however, in the basiscopic half of the segments at least, remains of a single cell in thickness in each, even at maturity.

We come now to the most important division of the soral segment, the basiscopic ultimate marginal cell (Figs. 34–38), from which are derived all the sporangia of the sorus. This is the 'Sorusmutterzelle' of Büsgen, but this name seems inappropriate as there is no single mother-cell of the sporangia of the sorus alone, nor of the whole sorus including the indusium after the single marginal cell of the third grade. No dermatogen-wall is formed in these marginal cells, and the sori being derived thus, like the young sporocarp itself, from a cell capable of forming at least two of the meristem-layers, are not of strictly epidermal origin. As the young sporocarp increases in size, we find that soon after the formation of section VI the basiscopic marginal cell elongates in the direction of the length of the organ, and divides by a transverse anticline into halves, of which the acroscopic one soon comes to be the larger (Figs. 34–38). Then each of these divides by another anticline (Fig. 38), forming thus four cells, of which the basiscopic one of the acroscopic pair soon becomes the largest (*p. ma-sp. m. c.*, Fig. 38), while its sister-cell on the acroscopic side splits by still another anticline. We have formed thus a series of five cells, of which the middle and larger one (*p. ma-sp. m. c.*, Figs. 34, 38) is the primary macrosporangium mother-cell giving rise to all the macrosporangia of the sorus. The adjacent cells on either side of the latter (*p. mi-sp., m. c.*, Figs. 34, 35, 38) are the primary microsporangium mother-cells, while the outer cells of the five (*i. ind.*, Figs. 34, 38) give rise to the inner layer of the indusium on each side. The outer layer of the indusium on each side is formed by the splitting in two of the acroscopic marginal cell by a transverse anticline (*o. ind.*, Figs. 35–38), one half helping to form the indusium of the sorus of its own segment, and the other of the sorus of the next younger segment.

In horizontal section it is seen that the three middle or sporangial cells become more densely filled with protoplasm than the indusial cells (Fig. 35), and also become separated at the ventral surface from the cells of section V (*s. c.*, Figs. 29, 35), thus forming the beginning of the soral canal. Otherwise the development of all of the five cells is much alike at first, and if we take transverse sections in the plane of the sporangial cells, we find that each elongates considerably in a radial direction (Fig. 29), and later divides into two by a pericline (Fig. 30). Then by the further growth and division of both of these cells (*ma.-sp. m. c.*, Fig. 31), there is formed a row of seven or eight cells reaching from about the centre of the capsule nearly to the ventral surface (Fig. 32), all of them separated by the soral cavity or canal from that part of the indusium formed from section V. In sagittal section (Fig. 43) it is seen that the microsporangial and indusial cells have divided in a similar manner.

From the occurrence in them of nuclear spindles and their relation to the surrounding cells, there can be no doubt that all the sporangial cells of the sorus are derived from the marginal cell, and none from cells dorsal to this in the interior of the capsule, as Büsgen thought possible. In the bending of the soral canal that takes place as it increases in length, the sporangial cells may come in contact with the inner layer of the indusium, but there is certainly no growing together, and the phenomenon has no significance. It is during the development of this row of soral cells that they are outgrown and finally enclosed by the surrounding cells, forming at first a 'funnel-like pit' at the ventral end of the soral canal (Figs. 30, 31), but finally closing entirely, though leaving traces of the fusion for a long time (*s. c.*, Figs. 32, 33).

While the sporangial and indusial cells have increased in numbers by the radial growth and division, there have been other important changes. The macrosporangium mother-cells (*ma-sp. m. c.*, Figs. 35, 36) are pushed by the growth of the plerome of section VI (*pa. b.*, Figs. 36, 37) out into the soral cavity, far beyond the microsporangium mother-cells, swell

laterally to several times their former size, and in so doing push the microsporangial cells around (Figs. 36, 37) to a position nearly at right angles to their former one. The macrosporangium mother-cell finally divides by three inclined walls to form the tetrahedral apical cell of the macrosporangium (*ma. sp.*, Figs. 32, 37). This apical cell cuts off two more segments on each of the three sides below (Fig. 41), which form the stalk and basal wall of the sporangium; then a pericline is formed near the outer end of the apical cell, cutting off the archesporium (*arc.*, Fig. 41) and completing the sporangium wall. The archesporium, as Russow has shown, then gives rise to the tapetum and spores.

While the microsporangial cells are being pushed aside as described above, each has divided by anticlines approximately parallel to the segment wall, first to two (Fig. 36) and then to four (Fig. 41). These come to lie parallel to the segments of the apical cell of the macrosporangium, and are evidently the cells which Russow supposed to be segments of this, but there can be no doubt that they are really derived as described above.

Of the four cells formed from each of the microsporangial cells as just described, the lower three go to form sterile tissue of the placenta (*pa.*, Fig. 41), while only the upper one, next to the macrosporangium, actually forms microsporangia. Each of these upper cells divides by walls transverse to the axis of the sorus to form four cells on each side of each macrosporangium (which are well seen in a sagittal section of a capsule somewhat older than that shown in Fig. 43). Then each of these four cells swells out from the placenta, and divides into a basal cell (*st. c.*, Fig. 41) and an outer cell, in which is formed later the tetrahedral apical cell giving rise to the stalk, walls, and archesporium. This basal cell of the microsporangium may be considered as homologous with the stalk-cell found in other Leptosporangiates, but nothing was seen in the development of the macrosporangium that could be regarded as such. In this latter respect *Marsilia* appears to differ from *Pilularia*, where Campbell ('93) states that such a cell is formed, at least occasionally.

In the development of the sporangial cells just described, the plerome of the acroscopic part of the basiscopic half of section VI has played an important part. The cells derived from this (*pa. b.*, Figs. 35, 36) push in back of the swelling macrosporangium mother-cell and between the placental cells (*pa.*, Figs. 37, 41) derived from the primary microsporangium mother-cells. Most of these cells derived from section VI form the middle portion or axis of the placenta, but a row of them next to the base of the macrosporangium (*pa. b.*, Fig. 36), and running the whole length of the sorus (*pa. b.*, Figs. 32, 33, 44), develop the vascular bundle of the placenta, while at a point about opposite the middle of the sorus these same cells become modified across the whole width of section VI (*pa. br.*, Figs. 31–33, 42, 44), to form the placental branch connecting the placental bundle with the lateral bundle.

During this activity of the other cells of the sorus the indusial cells have been developing also. The acroscopic part of section V and the acroscopic marginal cell have each split by a transverse anticline (Figs 35, 36) to form, in connexion with the cells of section II, the complete outer layer of the indusium (*o. ind.*, Figs. 35–37, 41, 43). The inner indusial cells derived from the basiscopic marginal cell and the basiscopic portion of section V (*i. ind.*, Figs. 32–37, 41, 43) complete the inner layer also. Each of these layers remains one cell in thickness throughout; but by growth of the cells in a direction parallel to, and division by walls perpendicular to the surface of the indusium, the latter pushes out so as to accommodate the growing sporangia. During the growth of the indusium intercellular spaces appear at many points between the two layers (Fig. 41), and other larger ones between the outer layers of the indusia of adjacent sori, both laterally and along the median wall (*i s.c*, Figs. 41, 42). By the increase in size of the latter spaces the indusia of adjacent segments become entirely separated, and the sori of each side of the capsule push into the furrows between the sori of the opposite side (Fig. 42) At a time a little

before this happens the sori may appear opposite each other, though they are really alternate in origin, as we have seen above (Fig. 34).

Finally we come to speak briefly of the last six or seven pairs of segments of the capsule, beyond the youngest soral segments. In these there is no single dorsal bundle, as this divides to two just beyond the origin of the lateral bundles of the last pair of sori. These two divisions run along nearly parallel to each other near the dorsal wall of the capsule (Fig. 44), and each gives off three or four branches which arise, like those in the soral segments, from the plerome of sections III and IV, and are joined like those also with their fellows of the same side near the ventral margin. The exact region of origin of the two divisions of the dorsal bundle was not made out satisfactorily. All the plerome of this region, except the little devoted to the dorsal and lateral bundles, is apparently devoted to the formation of the gelatinizing tissue of this part of the capsule.

Summary and Conclusions.

The leaves of *Marsilia* arise in two rows on the stem each from a cell quite near the growing-point. The two-sided apical cell formed in this leaf mother-cell cuts off fifteen pairs of segments, and these are divided by radial anticlines into six main divisions, five sections, and an ultimate marginal cell of the sixth grade. Four of these divisions on each side take part in the formation of the axial bundle of the petiole, while all of them help to form the mesophyll and epidermal tissues. One quarter of the vascular tissue contributed by section II develops without further division to the large trachea of its side of the bundle, which has its oblique end-walls always inclined in the same direction. Fourteen air-canals are formed between the mesophyll and hypodermis of the petiole, and a single longitudinal row of the mesophyll-cells gives rise to both the longitudinal and transverse partitions between

these. Another longitudinal row of the same cells gives rise to each of the tannin-sacs.

The pinnae or divisions of the lamina are formed by the continued activity of the marginal cells of certain segments, but their limits do not correspond exactly with those of the segments, the lower pair being nearly two segments in length and the upper pair about three.

In its mode of origin, then, the leaf of *Marsilia* agrees closely with that of other leptosporangiate Ferns, as it does also in its further growth by the segmentation of a two-sided apical cell. But the position of the first division-walls in these segments, while very like that described for *Asplenium serpentini* by Sadebeck ('74), is apparently quite unlike that described for *Ceratopteris* by Kny ('75), for *Onoclea* by Campbell ('87), and that given by Campbell ('95, p. 325) for the Leptosporangiates in general. In the development of the lamina also *Marsilia* is unlike other described forms except *Ceratopteris*, since the pinnae are not co-extensive with the segments as in *Onoclea* and *Asplenium*, though all agree in having the pinnae formed by the activity of a series of marginal cells. There is however great need of more detailed work on the origin of the leaf and the differentiation of this into petiole and lamina.

The sporocarp of *M. quadrifolia* is developed from a transversely placed apical cell, arising in a marginal cell on the inner side of the young leaf. The second sporocarp when present (usually) arises in the same way from a marginal cell of the first. The two are thus respectively primary and secondary branches of the leaf.

More rarely we may find two or more sporocarps inserted separately on the petiole, both on the same side. Then the suggestion is a tempting one, more especially in cases like *M. polycarpa*, where ten or more sporocarps may be borne in the same way, that the sporocarps represent pinnae homologous with those at the tip of the petiole, and the study of abnormal pinnae by Büsgen may perhaps seem to favour this. But before accepting this we have to account for the

occurrence of the sporocarps on one side of the petiole only, and also for their origin by a single apical cell instead of a series of marginal cells like the pinnae.

Growth by the apical cell continues till more than twenty pairs of segments are formed. In the primary division of the segments one more section is formed dorsal to the marginal cell than in the leaf. The epidermis is formed much as in the leaf, but the mesophyll and its air-canals are less developed, while the hypodermis is of two much-thickened layers. The longitudinal bundle (axial in the stalk and dorsal in the capsule) is derived from section I only; the lateral branches of this in the capsule are formed in sections III and IV; and the placental bundle and branch from section VI. The sporangia of each sorus are all derived from one macrosporangial cell, and two microsporangial cells are formed in the basiscopic marginal cell of each soral segment.

The microsporangia and the macrosporangia are thus derived from sister-cells, and the former do not come from segments of the apical cell of the latter as described by Russow and Büsgen; neither is the view of these authors as to the origin of the placental bundle from these same segments the correct one, as was stated a few lines above. A stalk-cell, homologous perhaps with that of the other Leptosporangiates, is formed in the development of the microsporangium, but nothing that could be so interpreted was seen in the macrosporangium.

The soral canals arise by the separation of the primary sporangial cells from the outer cells of section V, and are entirely external in origin. The indusium surrounding each sorus arises by the more rapid growth of the superficial cells of the ventral side of the capsule which grow out and close together over the ends of the sporangial cells. Its development thus seems to warrant the statement that it is a true indusium morphologically as well as physiologically. The gelatinizing tissue of the dorsal part of the capsule is apparently the equivalent of a part of the vascular tissue of the petiole, while that at the ventral edge probably comes

from the outer, and that at either end of the capsule from all three meristem-layers.

The walls of the capsule, including the vascular bundle-system, are developed entirely, or practically so, from the four sections in each segment dorsal to the marginal cell. Hence the two valves into which the capsule splits at bursting cannot be homologized with the divisions of the lamina, since these are developed from the numerous sections formed on both sides by the continued activity of the marginal cells. For this reason also any seeming similarity in the branching of the vascular bundle-systems of the two organs can have no meaning in the direction of homology.

We have here then another reason, in addition to the one mentioned above in speaking of the mode of origin of the sporocarp from the petiole, for not believing with Goebel that it represents a single leaflet or pinna with its edges folded in to meet at the ventral margin of the capsule. And the same objections hold against other views involving a belief in the laminar nature of the valves, such as that of Russow and Büsgen, who regard the capsule as made up of two leaflets with ventral surfaces facing each other, or that of Campbell and Meunier, who compare it to a folded pinnate leaf with a sorus for each pinna.

As far as developmental history gives any clue, the sporocarp of *Marsilia* is homologous with the petiole only of the sterile branch of the leaf. But before adopting this unreservedly we have to explain why there should be the marked difference in the development of the longitudinal vascular bundle in the two, especially in such very similar structures as the petiole and stalk.

So far as we have light at present, then, we may consider the capsule as the swollen end of a petiole in which the marginal cells are devoted to the formation of the sporangia instead of a lamina.

List of Writings Referred to.

Bischoff, G. W., '28: Die kryptogamischen Gewächse. Nuremberg, 1828.

Bower, F. O., '89: The Comparative Examination of the Meristems of Ferns. Ann. Bot., Vol. iii, 1889.

Braun, A., '70: Ueber Marsilia und Pilularia. Monatsb. Berl. Akad., 1870.

Büsgen, M., '90: Untersuchungen über normale und abnormale Marsilienfrüchte. Flora., Bd. lxxiii, 1890.

Campbell, D. H., '87: The Development of Onoclea Struthiopteris. Mem. Boston Soc. Nat. Hist., 1887.

———— '93: The Development of the Sporocarp of Pilularia Americana. Bull. Torrey Bot. Club, Vol. xx, 1893.

———— '95: The Structure and Development of the Mosses and Ferns. New York, 1895.

Goebel, K., '82: Ueber die 'Frucht' von Pilularia globulifera. Bot. Zeit., Bd. xl, 1882.

Hanstein, J., '62: Ueber eine neuholländische Marsilia. Monatsb. Berl. Akad., 1862.

———— '65: Die Befruchtung und Entwickelung der Gattung Marsilia. Pringsh. Jahrbücher, IV, 1865.

Hofmeister, W., '62: On the germination, development, and fructification of the Higher Cryptogamia, &c. Ray Soc., London, 1862.

Klein, L., '84: Vergleichende Untersuchungen über Vegetationspunkte dorsiventraler Farne. Bot. Zeit., Bd. xlii, 1884.

Kny, L., '75: Entwickelung d. Parkeriaceen dargestellt an Ceratopteris thalictroides. Nova Acta, &c., Bd. xxxvii, 1875.

Mettenius, G., '46: Beiträge zur Kenntniss der Rhizocarpeen. Frankfurt a. M., 1846.

Meunier, A., '87: La Pilulaire. Étude anatomico-génétique du Sporocarpe chez la Pilularia globulifera. La Cellule, tome iv, 1887.

Poirault, G., '90: Recherches d'histogénie végétale. Mém. de l'Acad. Imp. de St.-Pétersbourg, tome xxxvii, 1890 [1].

Prantl, K., '81: Untersuchungen zur Morphologie der Gefässkryptogamen, Heft ii, Die Schizaeaceen. Leipzig, 1881.

Russow, E., '72: Vergleichende Untersuchungen d. Leitbündelkryptogamen. Mém. de l'Acad. Imp. de St.-Pétersbourg, tome xix, 1872.

Sadebeck, R., '73: Wachsthum der Farnwedel. Verhandl. d. Bot. Vereins der Prov. Brandenburg. Bd. xv, 1873.

———— '74: Ueber die Entwickelung des Farnblattes. Berlin, 1874.

[1] I regret that Poirault's work did not come to my notice before the present paper went to press, so that I might have mentioned certain points in his work concerning the origin of tissues in the leaf of *Marsilia* which my own work confirms.

EXPLANATION OF FIGURES IN PLATES X, XI, AND XII.

Illustrating Dr. Johnson's paper on the Development of *Marsilia quadrifolia*.

Abbreviations used :—*A.*, direction of the apex ; *a. b.*, axial bundle of the petiole ; *a. c.*, air-canal ; *arc.*, archesporium ; *a. s. w.*, acroscopic segment wall ; *B.*, direction of base; *b. c. tc.*, basal cell of the trichome ; *b. m. c.*, basiscopic marginal cell ; *br.*, branch (axial bud) ; *b. r.*, rod of brown-walled cells ; *b. s.*, bundle-sheath ; *b. s. w.*, basiscopic segment-wall ; *b. w.*, basal wall of the capsule ; *c.*, capsule ; *c. p.*, transverse partition ; *c. p. c.*, transverse partition-cell ; *c. p. p.*, spores in transverse partition ; *D.*, dorsal side ; *d.*, dermatogen ; d^1., d^2., &c. dermatogen of sections I, II, &c. ; *d. b.*, dorsal bundle of sporocarp ; *d. w.*, dermatogen-wall ; *ep.*, epidermis ; *F.*, sporocarp ; F^1., first sporocarp ; F^2., second sporocarp ; *F. m. c.*, mother-cell of sporocarp ; *g. r.*, gelatinous ring ; *h. a.*, halving anticline of section I ; *hy.*, hypodermis ; *ind.*, indusium ; *i. ind.*, inner layer of indusium ; *i. s.*, intercellular space ; *i. s. c.*, inter-soral cavity ; *L.*, leaf ; *l. a.*, first longitudinal anticline in plerome of section V ; *l. b.*, lateral branch of dorsal bundle ; *l. b. f.*, fork of the lateral branch ; *l. p.*, longitudinal partition ; *l. p. c.*, longitudinal partition cell ; *l. p. p.*, pores in longitudinal partition ; *l. t.*, lower tooth ; *ma-sp.*, macrosporangium ; *ma-sp. m. c.*, macrosporangium mother-cell ; *m. c.*, marginal cell ; $m. c^1$., $m. c^2$, &c., marginal cell of the first, second, &c. grade ; *mi-sp.*, microsporangium ; *mi-sp. m. c.*, microsporangium mother-cell ; *mp.*, mesophyll ; *m. w.*, median wall ; *o, hy.*, outer layer of hypodermis of capsule ; *o. ind.*, outer layer of indusium ; p^1., lower pinna ; p^2., upper pinna ; *pa.*, placenta ; *pa. b.*, placental bundle ; *pa. br.*, placental branch ; pb^1., periblem of section I ; pb^2., periblem of section II ; *pb.*, periblem ; *p. c.*, partition-cell ; *pl.*, plerome ; pl^1., plerome of section I ; pl^2., plerome of section II ; *pl. w.*, plerome-wall ; *p. ma-sp. mc.*, primary macrosporangium mother-cell ; *S*, stem ; *sc.*, soral cavity ; *st.*, stalk ; *st. b.*, stalk-bundle ; *st. c.*, stalk-cell of microsporangium ; *s. w.*, segment-wall ; ta^1., ta^2., &c. first, second, &c. transverse anticlines of marginal cell ; *tc.*, trichome ; *tp.*, tapetum ; *tr.*, trachea ; *t. s.*, tannin-reservoir ; *u. t.*, upper tooth ; *V.*, ventral side ; *X.*, apical cell ; *I. II. III.*, &c. first, second, &c. section-walls ; 1. 2. 3., &c. first, second, &c. segments of the apical cell on one side.

All figures are camera drawings, and all are from microtome-sections, except Figs. 25, 31^a, and 44.

PLATE X.

Fig. 1. Transverse section of stem through apical cell of young leaf. × 300.
Fig. 2. Sagittal section of a leaf nearly at the end of apical growth. × 200.
Fig. 3. Part of a sagittal section of the petiole of an older leaf. × 300.
Fig. 4. Ventral surface of tip of a young leaf. × 400.

Fig. 5. Dorsal surface of the tip of a similar leaf. × 400.

Fig. 6. Half of a nearly transverse section of a young leaf showing the shape of a segment and the position of the first two section-walls. × 700.

Fig. 7. The same still older. × 750.

Fig. 8. Transverse section of petiole in which epidermal and hypodermal layers are completed and the partition-cells are nearly ready to cut off the cross-partition-cells. × 750.

Fig. 9. The same section of a still older petiole. × 400.

Fig. 10. Transverse section of a nearly mature petiole. × 60.

Fig. 11. Tangential section of petiole showing the air-canals and partitions. × 400.

Fig. 12. The same in a slightly older petiole. × 400.

Fig. 13. The same still later showing the lengthening of the longitudinal partition. × 400.

Fig. 14. Surface view of a longitudinal partition showing the pores in a nearly mature petiole. × 125.

Fig. 15. Surface view of petiole showing the arrangement of the trichomes. × 750.

Fig. 16. Nearly mature stage of same. × 300.

Fig. 17. Nearly mature trichome. × 60.

Fig. 18. Horizontal section of the tip of a leaf, showing the beginning of the first pinnae. × 400.

Fig. 19. Sagittal section of a leaf through one of the well-developed lower pinnae. × 400.

Fig. 20. Transverse section of a leaf through pinnae, a little later than Fig. 18. × 300.

Fig. 21. A similar section still later. × 125.

Fig. 22. A transverse section of petiole showing origin of sporocarp. × 750.

Fig 23. Part of an approximately horizontal section of base of a leaf showing the apical cell of sporocarp. × 750.

Fig. 24. Transverse section of stem, and a young leaf with two sporocarps, all three nearly parallel to the stem. × 150.

Fig. 25. Inner side of a young leaf with a sporocarp in which the segmentation of the apical cell is nearly finished. × 25.

PLATE XI.

Fig. 26. Nearly horizontal section of a stem through two leaves, an axillary branch and two sporocarp-rudiments, showing the under surface of part of older leaf, and cross-section of the first sporocarp arising on this; also the origin of a second sporocarp from the first. × 400.

Fig. 27. Transverse section of a young sporocarp. × 750.

Fig. 28. Transverse section through the basiscopic ultimate marginal cell of a sporocarp after all six sections are formed. × 750.

Fig. 29. The same section of a capsule at the time of beginning of soral canal, showing relative thickness of older and younger walls. × 750.

Fig. 30. Part of transverse section of older capsule. × 750.

Fig. 31. The same still older. × 400.

Fig. 31ª. A slightly older sporocarp than Fig. 25, on a petiole, showing capsule bent against the stalk.

Fig. 32. The same as Fig. 31 at the time of closing of soral canal. × 500.

Fig. 33. The same still later. × 45.

Fig. 34. Ventral surface of capsule a little older than Fig. 25. × 750.

Fig. 35. Part of horizontal section of about the age of Fig. 30 near the ventral surface. × 750.

Fig. 36. Horizontal section of capsule about the age of Fig. 31 near the ventral surface. × 750.

Fig. 37. The same of about the age of Fig. 32. × 750.

Fig. 38. Sagittal section through the marginal cells of capsule about the same age as Fig. 29. × 750.

Fig. 39. Sagittal section through sections III and IV of capsule, the same age as the last. × 750.

PLATE XII.

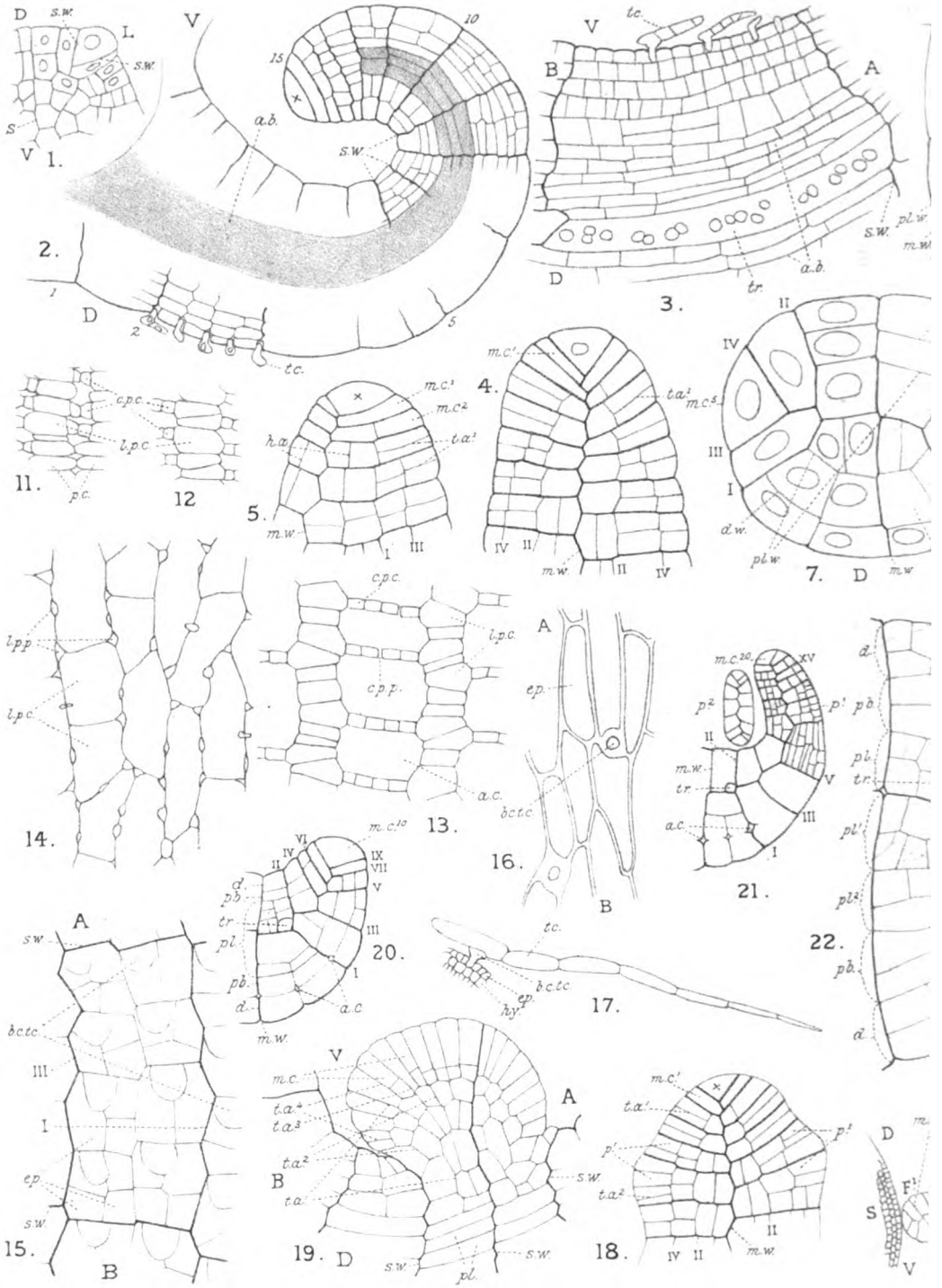
Fig. 40. Sagittal section through sections III and IV of a capsule of about the age of Fig. 31. × 750.

Fig. 41. Part of horizontal section of capsule of the age of Fig. 33. × 750.

Fig. 42. The same of whole capsule of same age as Fig. 41. × 50.

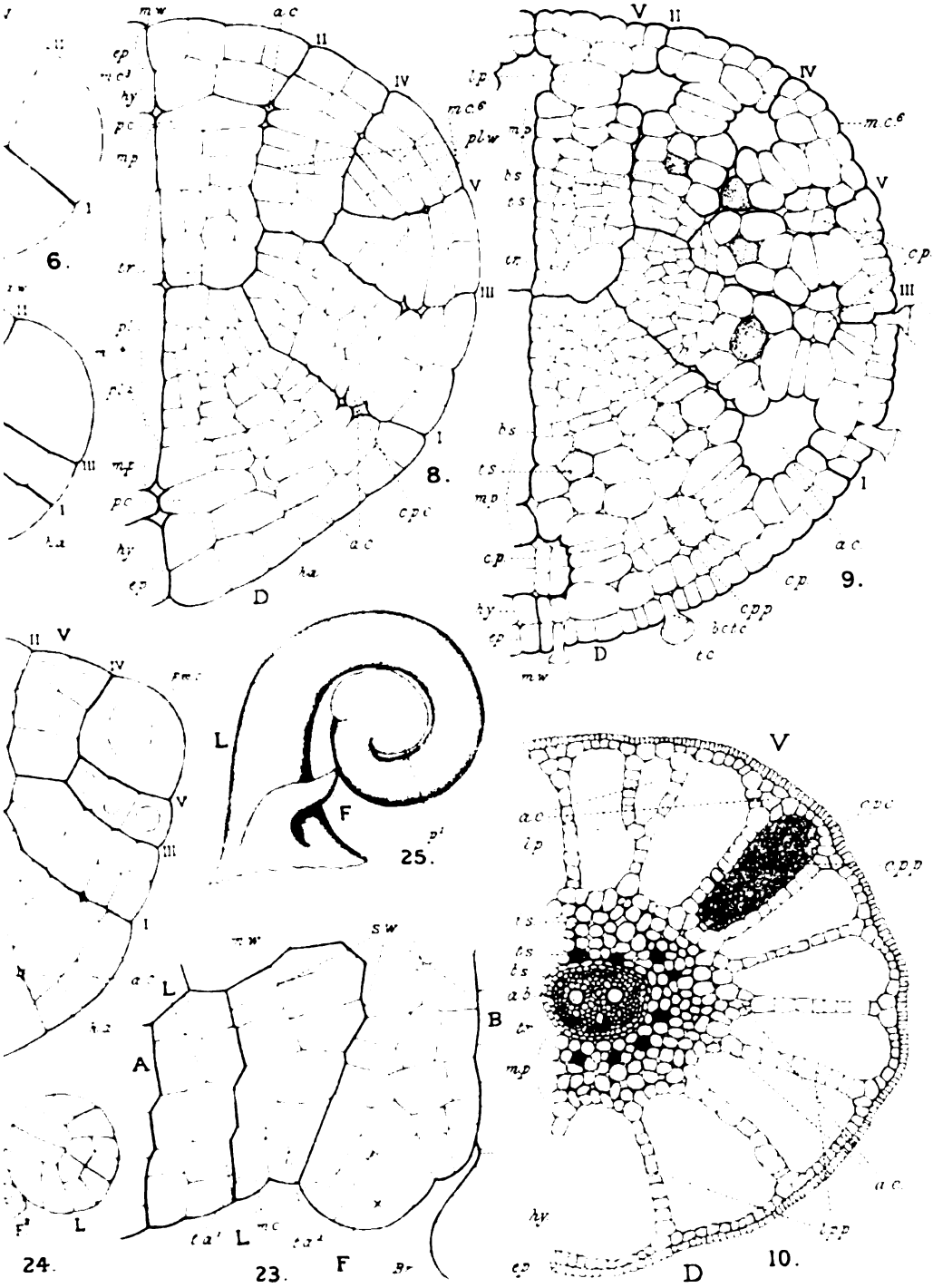
Fig. 43. Sagittal section through sori of same capsule as Fig. 40. × 750.

Fig. 44. View of inner side of one of the valves of a nearly mature capsule. × 8.

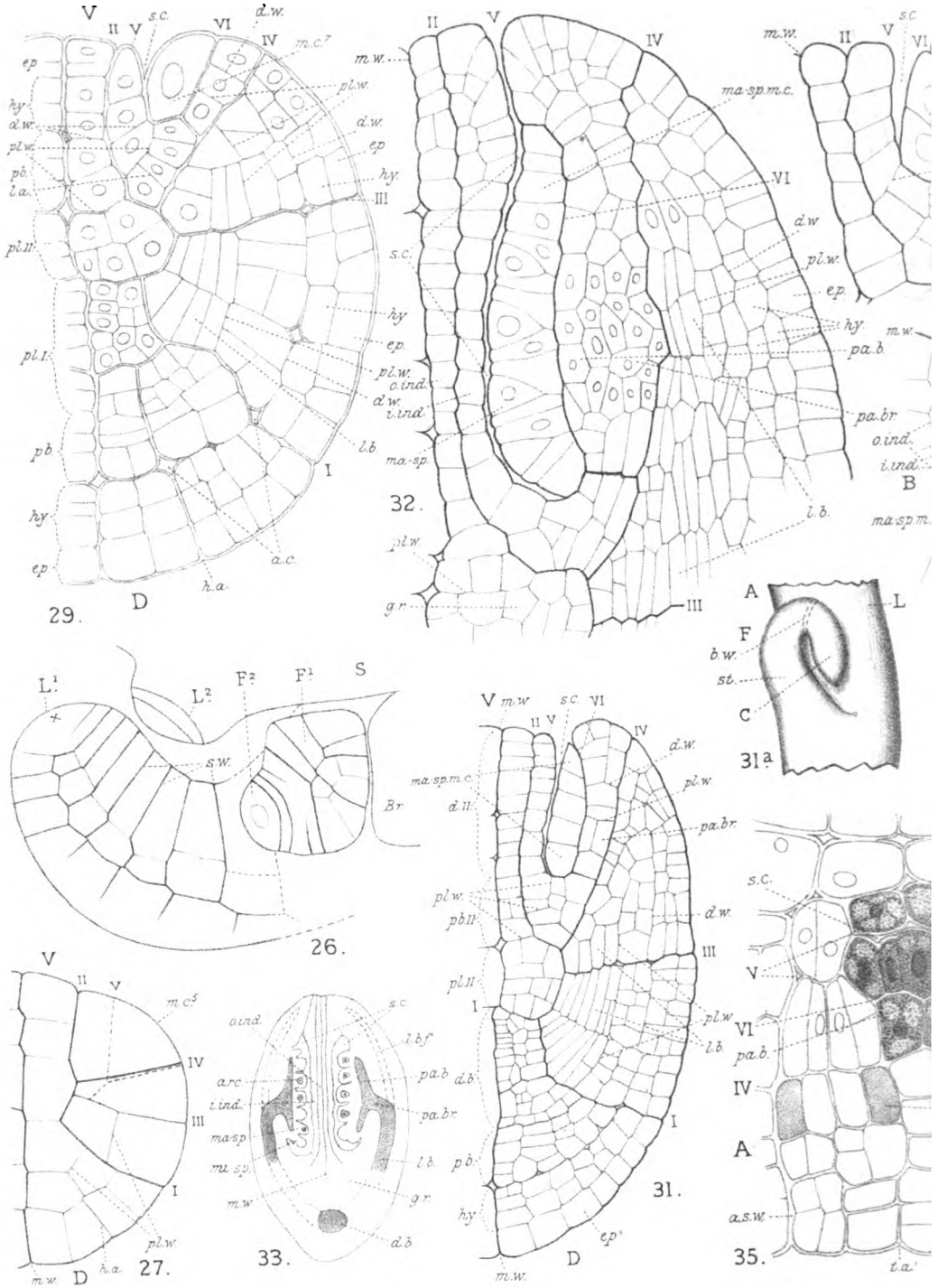


D S Johnson del

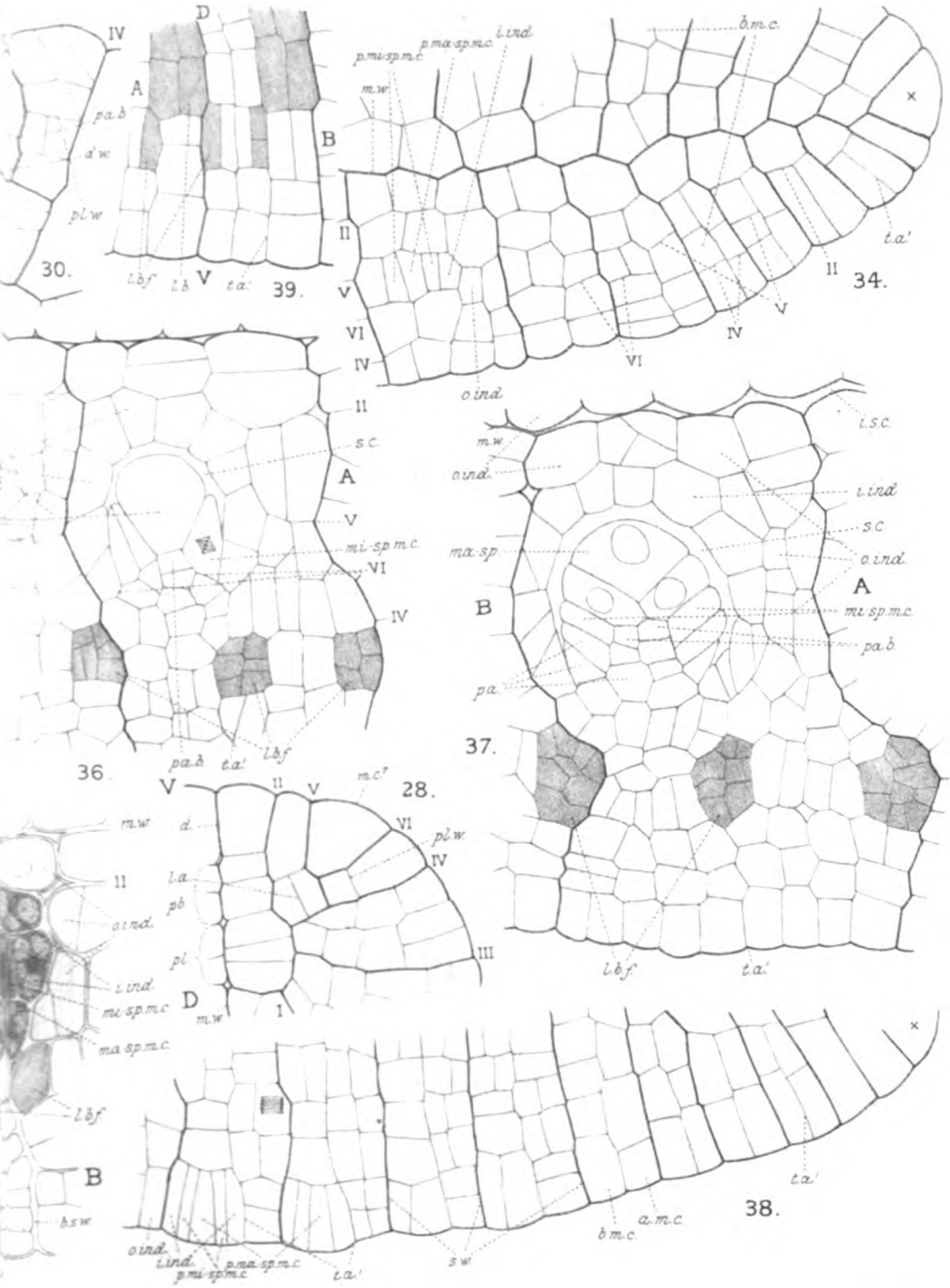
JOHNSON. — MARSILIA.



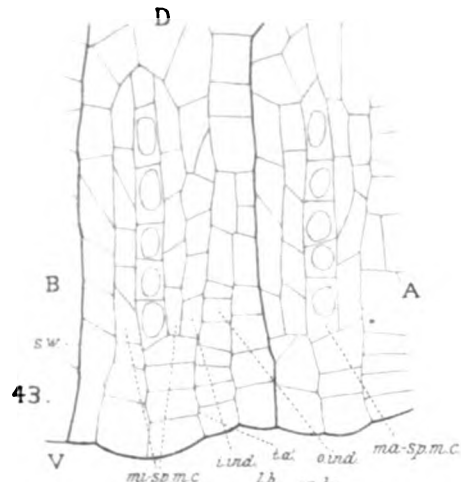
University Press, Oxford

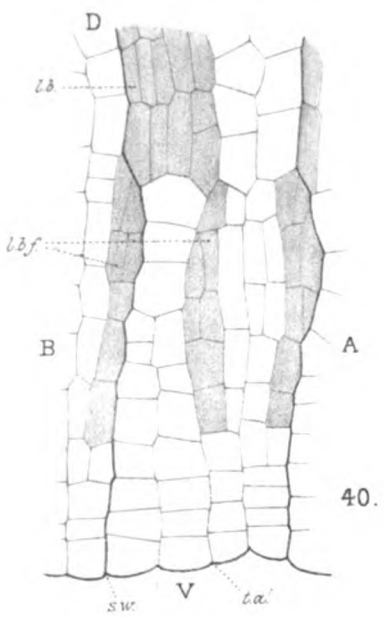


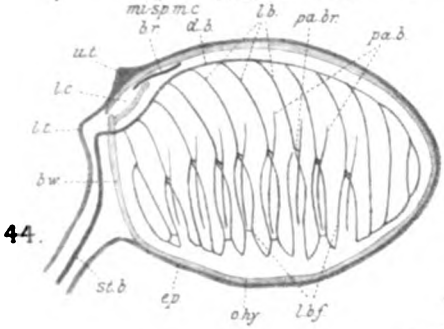
JOHNSON. — MARSILIA.

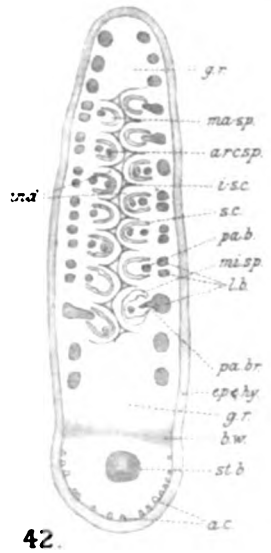


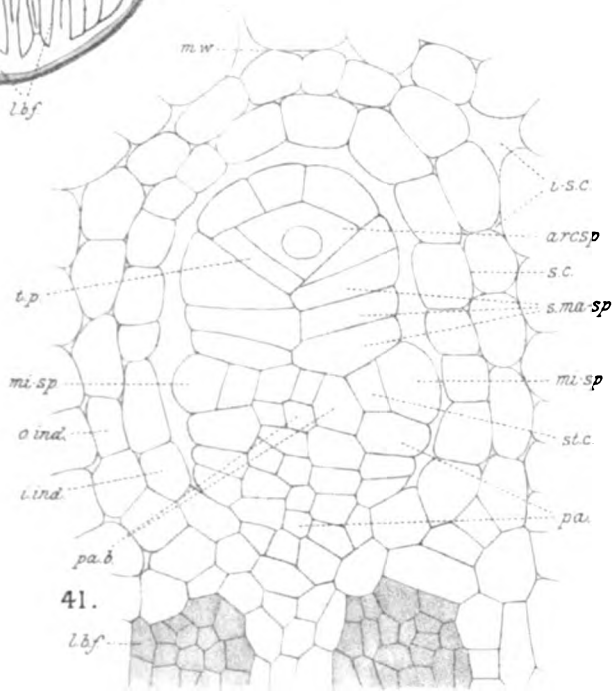
University Press Oxford











D.S. Johnson del. University Press, Oxford

JOHNSON. — MARSILIA.

On some points in the Histology of Monocotyledons.

BY

JOHN PARKIN, M.A.,

Trinity College, Cambridge.

With Plate XIII.

I. Observations on the Raphides (Figs. 1–12).

WHEN recently examining microscopically a number of Monocotyledonous leaves and reserve-organs for the purpose of investigating the occurrence and distribution of carbohydrates in them, I also paid some attention to the raphides so often present, and accumulated certain facts concerning them, some of which are apparently new. It was my intention to make a more complete investigation of this class of calcium-oxalate crystals; but at present, not having sufficient time at my disposal, it seems worth while to notify the few features that have come under observation.

The word 'raphides,' introduced first by De Candolle, is used to denote bundles of needle-shaped crystals which are arranged generally, but not necessarily, in a parallel manner. Each bundle arises in and occupies a single cell. Such cells I term raphide-cells. Although raphides occur in some Dicotyledons, they are pre-eminently characteristic of Mono-

[Annals of Botany, Vol. XII. No. XLVI. June, 1898.]

cotyledons, often existing in great abundance in the leaves, stems, and especially the reserve-organs. Raphide-cells are very constant in character, only differing from one another in size and in the length of the individual crystal. The bundle of raphides does not fill the cell-cavity, but is suspended in a mass of mucilage. The nucleus and protoplasm originally present in the developing cell disappear when it is fully formed, as Hilgers[1] showed in the case of *Polygonatum*. In tissue preserved in alcohol, the bundle is seen lying in the centre of the cell surrounded by a sheath composed of the precipitated mucilage.

In the genus *Iris* the typical raphide-cells are absent, but crystal-sacs of a different kind are present. These contain each a large acicular crystal, and possess nuclei and protoplasm, but no mucilage. They have been known for a considerable time, and have been investigated by Hilgers[1] in two species of the genus. De Bary[2] also refers to them. What I desire to point out is that intermediate stages occur in certain petaloid Monocotyledons between these two types of crystal-sacs; although in using the term 'intermediate,' I do not want it to be inferred that there is necessarily any genetic relationship between the two. The five cases which I have observed are found in *Funkia ovata*, *Convallaria majalis*, *Phormium tenax* var. *atropurpurea*, *Tritoma Uvaria*, and *Polianthes tuberosa*.

Funkia ovata. Raphide-cells of the usual large type exist in fair abundance in the leaf-lamina and petiole (Figs. 1 and 2). Besides these, however, there is another kind of crystal-cell, which is more widely distributed in the plant, occurring in the leaves and root-stock. They might, without special attention, be passed over as ordinary raphides. The petiole is a good region in which to observe these two forms. By carefully examining transverse and longitudinal sections the differences between the two become very manifest.

[1] Hilgers, Pringsheim's Jahrbücher, Vol. vi, 1867–68, p. 285.

[2] De Bary, Comparative Anatomy of Phanerogams and Ferns, p. 138: he refers to Unger's Anatomie und Physiologie, 1855.

In a transverse section certain of the crystal-cells are seen to be of smaller diameter, to contain fewer individual crystals of greater sectional area, and to be without the envelope of mucilage (Fig. 3). They are arranged rather differently, being situated near the xylem, one to three being seen in a section to each bundle; odd ones also occur in the ground-tissue along with the ordinary raphide-cells.

In longitudinal section, the differences are more apparent. The new form of crystal-sac is narrower, and contains from ten to twenty needles of a larger size than the numerous ones composing the raphide-bundles. This type of crystal-sac also possesses both nucleus and protoplasm (Fig. 4).

The raphide-cell averages about 250 μ in length, and the individual raphide about 60 μ. The crystal-sac is very little longer than the crystals themselves, which average 100 μ., about double the length of the raphide.

Convallaria majalis resembles *Funkia* closely as regards its crystals, possessing in its leaves both ordinary raphide-cells, and sacs containing larger and fewer needles without mucilage. The latter are the more numerous. The roots and rhizomes seem only to have the mucilaginous raphide-cells. Hilgers[1] examined the mucilage of the raphide-cells, but makes no mention of the other kind of crystal-sac. In *Polygonatum multiflorum*, however, I have not succeeded in finding any type of crystal-cell other than the mucilaginous raphide cells.

Phormium tenax var. *atropurpurea*. This plant furnishes a more interesting case. In the leaf the crystals which attract attention are very similar to those found in *Iris*, being solitary prisms in cells without mucilage arranged in longitudinal rows (Figs. 5 and 6). Careful observation, however, also shows here and there ordinary raphide-cells. These are very sparsely scattered. The others are comparatively common, and odd ones contain two crystals instead of the usual solitary one (Fig. 7).

[1] Hilgers, loc cit.

Tritoma Uvaria. In this plant none of the ordinary mucilaginous raphide-cells could be found either in the root, stem, or leaf; but the other kind are numerous, varying considerably in size, from 60 μ. to 160 μ. in length. The crystals, which almost fill each sac, are fairly abundant. Nuclei and protoplasm can also be made out in them (Figs. 11 and 12).

Polianthes tuberosa. The tuber only has been examined. and in it are found both kinds of crystal-cells. The mucilaginous ones are more numerous and larger (Fig. 10). The others occur chiefly near the vascular bundles (Figs. 8 and 9).

From the fact that these two kinds of crystal-sacs occur in the same plant and even in the same tissue, it looks as if they may have arisen independently, and not have been derived the one from the other. Nevertheless the above instances suggest that as the raphide-cells decrease in number their place is taken by the other type.

In the order Iridaceae raphides seem wholly wanting, only the solitary crystals being present. Besides species of *Iris*, I have observed these prisms in the genera *Crocus*, *Sparaxis*, *Schizostylis*, *Xiphium*, *Freesia*, *Babiana*, *Gladiolus*, and *Montbretia*; and thus they appear characteristic of the order. It may be that the non-mucilaginous crystal-sacs discovered in *Funkia*, *Convallaria*, *Phormium*, *Tritoma*, and *Polianthes* are genetically connected with one another and with those of the Iridaceae.

The distribution of special crystal-cells in the large petaloid family, the Liliiflorae, is instructive. The tribes Tulipeae and Allieae are exceptional in containing none[1]. They seem very scarce in the Colchicaceae; *Veratrum* possesses a few raphide-cells, but I failed to find any in *Colchicum* or *Uvularia*. The Iridaceae, as previously shown, possess the large solitary needles. The other groups, as far as I know, contain the ordinary mucilaginous raphide-cells, such as the Hyacintheae, Anthericeae, Yuccoideae, Hemerocallideae,

[1] De Bary, loc. cit. p. 142.

Convallarieae, Asparageae, Dracaeneae, Pontederiaceae, and Amaryllidaceae. Then in addition to the ordinary raphide-cells, *Funkia*, *Convallaria*, *Phormium*, and *Polianthes* possess crystal-sacs without mucilage; while *Tritoma* appears to have the latter only.

Funkia, *Phormium*, and *Tritoma* belong to the tribe Hemerocallideae; *Hemerocallis fulva* and *H. flava*, both of which I have examined, contain ordinary raphide-cells, but not the other type of crystal-sac. The Convallarieae come very near the Hemerocallideae in habit, differing chiefly in the baccate fruit. Thus the four genera, *Funkia*, *Phormium*, *Tritoma*, and *Convallaria* seem fairly closely allied. *Polianthes* is placed among the Agaveae, a sub-order of the Amaryllidaceae. It is generally considered that the Amaryllidaceae have been derived from the Liliaceae by the ovary becoming inferior, and sometimes it is inferred that the Iridaceae have arisen from the Amaryllidaceae. It may be that the Iridaceous forms began to appear just about the time the Amaryllideae were evolving, both having a common origin in some Liliaceous type. The similarity of the crystal-sacs would support a relationship between the Iridaceae and the Liliaceous tribe Hemerocallideae.

A special study of these crystals, which are formed in the growing organs, and hence belong to Schimper's class of primary calcium oxalates, might be of value from a phylogenetic point of view, as well as a means of throwing light on their function, of which at present we seem very ignorant.

II. An absciss-layer in the leaves of *Narcissus*, *Galanthus*, and *Leucojum* (Figs. 13, 14).

Having had occasion to examine species of *Narcissus*, *Galanthus*, and *Leucojum* at various stages in their annual growth, I observed that the foliage does not simply die down and wither away, but that each leaf is detached from its tunicate base (bulb-scale) by means of a layer of cells

becoming merismatic. Not finding in botanical literature any description of such an absciss-layer in these plants, it seems worth while making a note of it, and pointing out a few details connected with the occurrence.

Von Mohl[1] in his researches on 'leaf-fall' in reference to Monocotyledons, mentions merely the falling of their perianth-segments and immature capsules.

Bretfeld[2] has investigated the mode of detachment of leaves in *Dracaena*, many of the Orchidaceae and Aroideae, and generalizes from the study of these plants, that, whereas the leaf-fall in Dicotyledons is brought about by a new tissue formed a short time before the shedding of the foliage, in Monocotyledons it results from the action of a special mechanism, produced along with the other tissues in the developing leaf, similar to the contrivance which brings about the dehiscence of dry pericarps. However, the method here observed resembles that of Dicotyledons, although it is perhaps simpler in detail.

Some time before the leaves turn yellow, certain of the parenchymatous cells situated a little way above the tunicate base of the foliage-leaf become merismatic, and divide to form a zone of narrow cells with conspicuous nuclei and abundant protoplasm; this region is visible to the unaided eye as an opaque line on holding the leaf up to the light (Figs. 13, 14). In *Galanthus nivalis* the cell-divisions were just commencing when examined on April 16, and were well advanced on May 1; by the end of May the leaves, having turned completely yellow, are easily detached by means of their absciss-layers from their swollen bases, which now become the scales of the bulb, full of reserve material. The epidermal and mesophyll-cells and nucleated cells belonging to the vascular bundles take part in the divisions; the raphide-cells and, of course, the vessels remain passive, their lumina becoming obliterated by the pressure exerted on them by the adjacent dividing cells. As a rule, about four or

[1] Mohl, Botanische Zeitung, 1860.

[2] Bretfeld, Pringsheim's Jahrbücher, xii, 1880.

five irregular layers of small narrow cells result from the activity.

In these plants, besides the perfect foliage-leaves, there are sheathing phyllome-structures external to them, the lower parts of which also swell to form reserve bulb-scales, while the upper parts remain membranous and afford a covering to the active region of growth in the young foliage leaves. These upper sheathing parts are likewise divided off from their lower reserve-storing portions by the formation of absciss-layers. These in the case of *Galanthus nivalis* are well formed by the end of March, that is some time before those of the foliage-leaves.

The plane of detachment is through the middle of the absciss-layer, and previously to the separation the walls of the newly-formed cells become suberised, giving a deep yellow coloration with iodine and sulphuric acid, whereas the other cell-walls stain blue.

The plants in which these absciss-layers have been noticed by me are *Narcissus Pseudo-narcissus* and *N. poeticus*, *Galanthus nivalis*, *Leucojum vernum* and *L. aestivum*. No doubt other species of these genera exhibit them, and most likely other bulbous Amaryllideae.

Such an absciss-layer, by means of the corky walls formed and by the closure of the vessels, may possibly be a protection against the entrance of Bacteria or fungus-hyphae into the scales, and also a check to the passage of water out of them.

BOTANICAL LABORATORY, CAMBRIDGE.

EXPLANATION OF FIGURES IN PLATE XIII.

Illustrating Mr. Parkin's paper on the histology of Monocotyledons.

Figs. 1–12 taken from sections, cut from spirit-material and mounted in alcohol, so as to retain the mucilage in the cells. Drawn under a Reichert No. 5, objective.

Funkia ovata—petiole.

Fig. 1. Transverse section of a raphide-cell.

Fig. 2. Longitudinal section of the same: *r.* bundle of raphides; *m.* mucilage precipitated by alcohol.

Fig. 3. Transverse section of a crystal-sac with mucilage.

Fig. 4. Longitudinal section of the same: *c.* bundle of acicular crystals; *n.* nucleus; *p.* protoplasm.

Phormium tenax var. *atropurpurea*—leaf.

Fig. 5. Transverse section of a crystal-sac without mucilage.

Fig. 6. Longitudinal section of the same.

Fig. 7. Transverse section of a crystal-sac with two crystals: *c.* solitary crystal.

Polianthes tuberosa—tuber.

Fig. 8. Transverse section of a crystal-sac without mucilage.

Fig. 9. Longitudinal section of the same.

Fig. 10. Longitudinal section of a raphide-cell: *r.* bundle of raphides; *c.* bundle of acicular crystals; *n.* nucleus; *p.* protoplasm.

Tritoma Uvaria—root-stock.

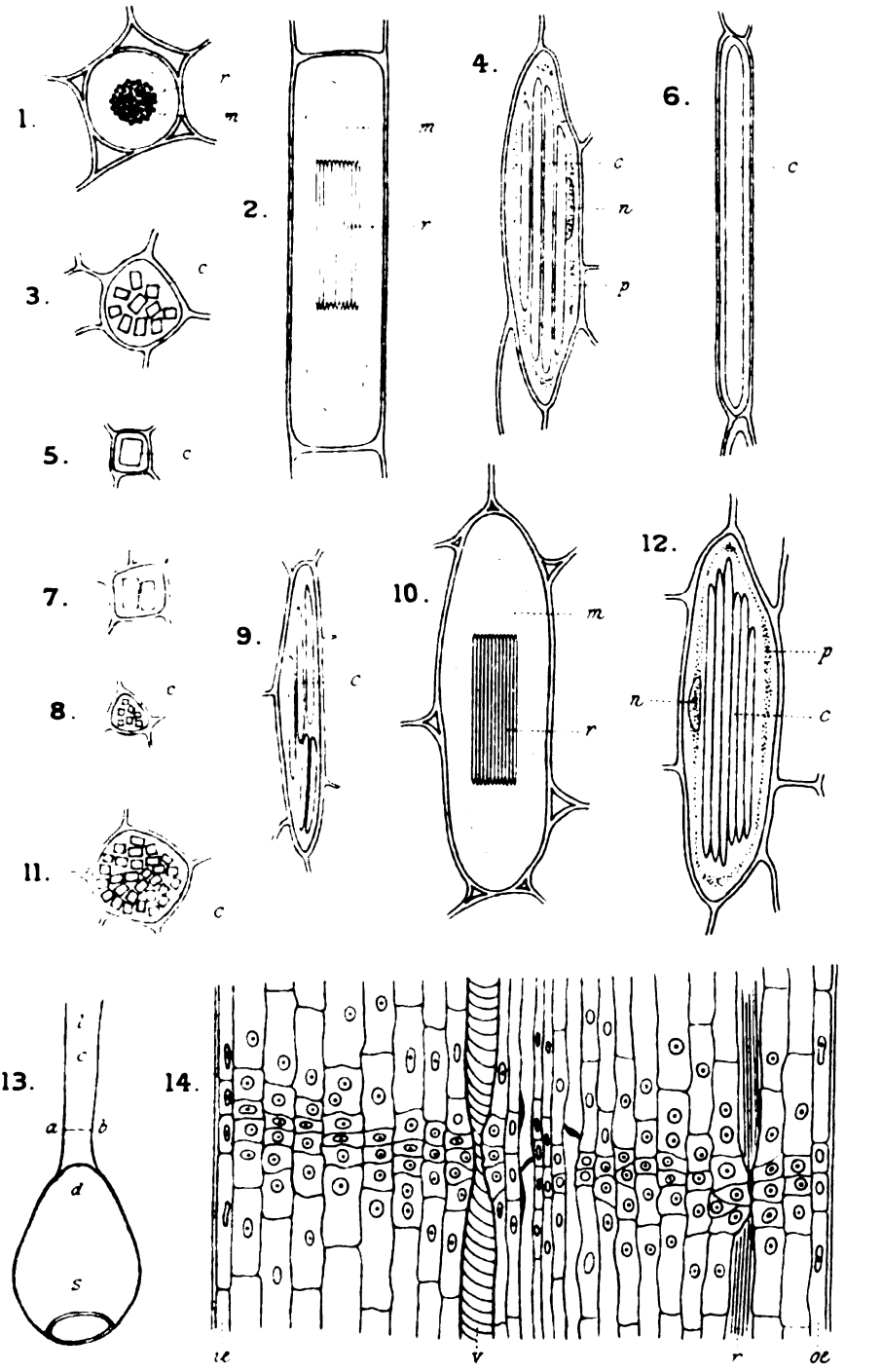
Fig. 11. Transverse section of a crystal-sac.

Fig. 12. Longitudinal section of the same: *c.* bundle of crystals; *n.* nucleus; *p.* protoplasm.

Narcissus poeticus—foliage leaf-base.

Fig. 13 (natural size). Line *a–b* shows the position of the absciss-layer; *l.* upper leaf; *s.* bulb scale.

Fig. 14. Semi-diagrammatic longitudinal section through region marked *c–d* in Fig. 13. The absciss-layer in process of formation is shown by the zone of narrow cells recently formed with conspicuous nuclei: *v.* vessel; *r.* raphide-cell; the lumens of both being obliterated by the pressure of the dividing cells; *oe.* outer or lower, *ie.* inner or upper epidermis.



University Press, Oxford

PARKIN. — MONOCOTYLEDONS.

On Aecidium graveolens (Shuttlew.)[1].

BY

P. MAGNUS, Berlin.

With Plate XIV.

IT was stated by Jacob Eriksson in his work, Studien über den Hexenbesenrost der Berberitze (*Puccinia Arrhenatheri*, Kleb.[2]). that the mycelium of the Rust of the Witches' Brooms of *Berberis vulgaris* (which had been named *Aecidium magellanicum*, Berk., in consequence of my remarks on the subject in Hedwigia, 1876) grows in the interior of the cells of the cambium of the witches' broom. In a paper published in the Berichte d. Deutsch. Bot. Ges., Vol. xv, 1897, pp. 148–152, I questioned these observations, and showed that an intercellular mycelium occurs in the branches of this plant in the pith, in the cortex and in the phloem, which sends out knot-shaped haustoria into the neighbouring cells; but I was unable to observe the intracellular mycelium described by Eriksson.

In a subsequent paper[3] this author criticized my results, stating that he had observed only the cambium, while I had given most attention to the pith and the tissues of the cortex, and therefore my observations counted for little as regards

[1] Read before Section K of the British Association, Toronto, 1897.
[2] Cohn, Beiträge zur Biologie der Pflanzen, Vol. viii, Heft I.
[3] Berichte der Deut. Botan. Ges., Vol. xv, 1897, pp. 228–231.

[Annals of Botany, Vol. XII. No. XLVI. June, 1898.]

the cambium. As I had, however, looked for the mycelium in *all* the tissues of the stem, as is evident from my paper, 'Ueber das Mycelium des *Aecidium magellanicum*, Berk[1],' this criticism is of little weight. Eriksson in fact admits that I had investigated the cambium when he quotes my statement that I did not find the mycelium in it. He further states that while he had worked with living material, I had only examined specimens preserved in alcohol. I do not regard such an objection as a reasonable one; nevertheless this year I have expressly used living material, and have obtained the same results. I have also extended my observations to ascertain how and where the mycelium develops in the branches of the witches' brooms, how it arises in the buds, and how it develops in the leaves which, even in the bud, are covered with the fructification of the Fungus.

For these observations I was able to use material rich in the fungus which was sent to me by Hofgärtner Reuter from the Pfaueninsel near Potsdam, on April 24, 1897, and by Herr J. A. Bäumler, from Pressburg in Hungary, on May 8, 1897. I owe my best thanks to these two gentlemen for this material. The latter was especially valuable to me, inasmuch as it consisted of a large number of young buds growing into elongated branches.

Of course I was not able to decide, in response to Eriksson's wish, whether this mycelium is derived from aecidiospores or from sporidia of germinated teleutospores. Up to the present time we do not know of any observations supporting the idea that such mycelia are different in any respect except in their extension in the host-plant. I have shown in earlier papers that the mycelium developed from the sporidia of the hibernated teleutospores often extends further into the tissue of the host-plant than that derived from the germinating uredospores or aecidiospores, which form in most cases a mycelium restricted to the region of infection (e.g. *Puccinia Oreoselini*, Strauss., and *P. Cyani*, Pass.): but no other

[1] Berichte der Deut. Bot. Ges., Vol. xv, 1897.

difference in the relation of these mycelia to the tissues of the host-plant has ever been observed. Therefore I cannot accept Eriksson's hypothesis that the mycelium of the aecidiospores can bear any relation to the tissues of the branches of the witches' broom, differing from that of the mycelium developed out of the germinating sporidia of the teleutospores.

The investigation of the fresh material, as I have already mentioned, has confirmed my earlier observations. The hyphae are always intercellular, and put out knot-shaped haustoria into the cells between which they grow. They occur in the pith, in the cortical parenchyma, in the phloem, and in the medullary rays (I had forgotten to mention the last in my communication of February, 1897). In the medullary rays the walls of the cells on which the hyphae grow are swollen (Figs. 7–9), and the hyphae send out numerous knot-like haustoria into the parenchymatous cells. I could not be certain that haustoria are also sent out into the wood-cells bordering on the medullary rays. I believe that this does take place, but I am not satisfied that the cells in which it seems to occur are not medullary ray-cells. In the elongated cells of the phloem the branches of the haustoria are sometimes not coiled up into a knot, but lie free in the cell (Fig. 6). The branches are not straight, but are curved or crumpled, and constricted here and there. As I have described earlier (*loc. cit.*), the mycelium is formed in the young branches of the witches' broom in the pith, in the medullary rays, and in the primary cortical parenchyma. In the latter it is often cut off from the surrounding tissue by the formation of cork in the infected tissue, so as to form island-like masses in the cortex [1].

Out of the primary cortical parenchyma in the older twigs the mycelium grows into the phloem and gradually penetrates into it as development proceeds. In the phloem also the infected parts of the tissue are often enclosed as islands by the formation of cork. These ring-like formations of cork

[1] Vide Berichte der Deut. Bot. Ges., Bd. xv, 1827, Taf. iv, Fig. 5.

give a very characteristic appearance to the transverse sections of the older stems of the witches' broom. In these mycelial hyphae, which have a very small lumen, I have never observed the yellow colouring-matter of the Uredineae described by Eriksson (*loc. cit.*) in the mycelial filaments of the cambium, which he states are intracellular. I will at once remark that I have generally observed the yellow colouring-matter of Uredineae only in the mycelial filaments which are exposed to the light, never in those portions of the mycelium which are embedded so deeply in the tissue of the host-plant as not to be exposed to the light.

As I have already stated elsewhere[1], the spermogonia appear on the whole surface of the first leaves, which are developed in April and the beginning of May, and the aecidia appear between the spermogonia; on the later leaves of the infected buds are found single larger or smaller groups of aecidia only, while the latest formed leaves are altogether free from the Fungus. At the end of April or the beginning of May a large number of these buds had already put out branches with long internodes. The leaves of these branches are free from the Fungus, as I have said. If longitudinal sections of these long branches be examined at the end of April or beginning of May, the hyphae in the pith will be seen growing in a longitudinal direction into the region of the merismatic tissue (Fig. 1). These mycelial strands are intercellular, and occur in the actually dividing cells of the parenchyma of the pith. The cells around these mycelial strands are sometimes more elongated than the others, and remain for some time in this condition, while the neighbouring cells are undergoing transverse division (Figs. 2, 4). This intercellular mycelium often sends into these neighbouring parenchymatous cells haustoria of the same kind as those already described (Figs. 2, 4). From these longitudinal mycelial threads horizontal threads grow out laterally (Fig. 2). These are the threads which grow into the medullary rays,

[1] Verhandl. des Botan. Vereins der Provinz Brandenburg, Sitzungsberichte, 1875, pp. 87–89 (which was also published in Hedwigia, 1876, No. 1).

and especially into the spaces between the vascular bundles where the leaves come off. These mycelial strands grow next spring into the buds which are formed at this place, and form spermogonia and aecidia over the whole surface of their leaves.

I will here shortly recapitulate these observations. The mycelium always grows between the cells, and gives off haustoria into them. In the first spring the hibernating mycelium grows into the developing buds, and forms spermogonia and aecidia on the whole surface of the first leaves. In the case of those short shoots which grow out into branches with long internodes, the mycelium grows directly into the pith and continues to grow with the merismatic tissue. This also takes place in the spring. From these medullary mycelial strands branches grow outwards, but these do not penetrate into the leaves. They pass through the medullary rays into the primary cortical parenchyma, and especially through the openings in the vascular cylinder, where the young leaves are given off to the axillary buds, from whence, in the following spring, the mycelium enters into the first developing leaves. As the branches of the witches' broom increase in thickness, the mycelium spreads from the primary cortical parenchyma into the phloem. Both in the primary cortical parenchyma and in the phloem the rows of cells affected by the mycelium are enclosed more or less completely by a cylindrical cork-formation, and are thus separated from the less affected tissue.

What, then, is the tubular mycelium in the cambium-cells described and figured by Eriksson (*loc. cit.*, Fig. 5, Pl. II), containing, contrary to what is observed in the mycelial strands of Uredineae growing in tissues not exposed to light, the yellow colouring-matter of the Uredineae? Leaving aside for the present the yellow colouring-matter, his figure resembles more than anything else the young cells of the cambium with horizontal transverse walls, the contents of which are contracted by plasmolysis. It is often the case in elongated cells that the plasmolyzed contents are for the

most part retracted only from the longitudinal walls, while remaining more or less in contact with the short horizontal or oblique walls (Fig. 10). These contracted cell-contents then resemble a tube placed longitudinally in the empty cells, and if the plasmolyzed contents are not retracted from the common transverse wall of neighbouring cells, they present the appearance of a continuous tube passing through the cell-cavities. Eriksson's figure appears to me to strongly suggest such plasmolytically contracted cell-contents, although it must be borne in mind that I have not examined Eriksson's preparations.

It is well known that the wood of the young Barberry is yellow, and that this is caused by the yellow cell-sap which is found even in the young wood-cells, thus indicating that the colouring-matter exists in a state of solution, and not in the form of yellow granules. The yellow colouring-matter of the granules observed by Eriksson in the tubes, which appear to me to be nothing more than plasmolyzed cell-contents, may be connected in some fashion with the colouring-matter of the young wood-cells. In making preparations, if the cells are cut across, the yellow sap flows out into the water, and then they appear colourless. The granules of the young wood-cells do not appear yellow. I cannot decide whether the yellow tint of the granules figured by Eriksson may not be due to the colouring-matter of the wood-cells seen through the cambium-cells (especially as Eriksson mentions in a foot-note to his paper in the Ber. d. Deutsch. Bot. Ges., Vol. xv, 1897, p. 229, that *all* the granules appeared yellow, and not as the lithographer has by mistake indicated, a few of them only), or whether they represent the first appearance of the yellow colouring-matter of the wood-cells. In no case have I been able to observe in the mycelium of any of the Uredineae not exposed to the light any trace of yellow colouring-matter. Even in the mycelium of the cortical parenchyma and the phloem no colouring-matter is found.

It seems to me that these results are of special interest, because Eriksson has lately maintained the theory of the

'mycoplasmastadium' of Uredineae[1]. In the case under discussion we can observe in succession the mycelium itself entering the buds, wintering there, and the following spring penetrating into the young leaves of the sprouting shoots, and there developing organs of fructification. The annual development of this parasitical Fungus does not exhibit the 'mycoplasmastadium' of Eriksson. There is no ground here for such a theory.

In conclusion I will give a short account of the identification of the European species producing the witches' broom on Barberry. In 1875 and 1876, when I had distinguished the aecidium producing the witches' broom of *Berberis vulgaris* from the aecidium of *Puccinia graminis*, I at first thought I had discovered a new species; but I found afterwards, to my great surprise, that Berkeley had described in Hooker's Flora Antarctica, II, pp. 450–451, a similar aecidium on *Berberis ilicifolia*, Forst., from the Straits of Magellan, and then I identified it as *Aecidium magellanicum*, Berk. On the ground of the similarity of appearance of the parasite, I believed that the aecidium of the witches' broom in Europe was identified with this aecidium of Berkeley, and accordingly I named the fungus causing the witches' broom of *Berberis vulgaris*, *Aecidium magellanicum*, Berk. All the later authors have followed me. Subsequently this identification appeared to me doubtful, as the cultures of Peyritsch and Eriksson had demonstrated that *Puccinia Arrhenatheri* (Kleb.), Eriks., (=*P. magellanica*, Peyr.) on *Arrhenatherum elatius* belongs to the aecidium of the witches' broom of *Berberis vulgaris*, whilst no *Arrhenatherum* or *Avena* occurs in Patagonia. In the Berichte der Deutsch. Bot. Ges., Vol. xv, 1897, pp. 270–276, I have shown that in Patagonia and Chile another aecidium occurs on *Berberis buxifolia*, Lam., which causes witches' broom, and which is well distinguished from the European species by the remarkable nestlike formation of

[1] See 'Vie latente et plasmatique de certaines Urédinées,' Comptes Rendus, 1897. Mars, and 'Der heutige Stand der Getreiderostfrage,' Berichte der Deut. Bot. Ges., Bd. xv, 1897. pp. 192–194.

its growth on single nodes, by the withering of the branches, by the swelling of the nodes which bear the witches' brooms, and by the absence of spermogonia. I have called this species *Aecidium Jacobsthalii Henrici*, P. Magn. It differs also from the *Aecidium magellanicum*, described by Berkeley on *Berberis ilicifolia*, Forst., which may be a third species attacking *Berberis*. The European Fungus forming the large witches' broom on the Barberry, with a great many elongated erect branches, can therefore no longer be called *Aecidium magellanicum*. It may be designated either as *Aecidium graveolens*, Shuttlew., which name was found by Cooke in the Paris Herbarium, or as the aecidium of *Puccinia Arrhenatheri* (Kleb.), Eriks.

I am deeply indebted to Mr. Harold Wager for having translated my German manuscript into English.

EXPLANATION OF FIGURES IN PLATE XIV.

Illustrating Prof. Magnus' paper on *Aecidium graveolens*.

The figures which accompany this paper were drawn from nature in my presence by Dr. Paul Roeseler.

Fig. 1. Longitudinal section of apex of spring shoot (in May, 1897) developed from a bud of witches' broom. At M. M. the mycelium is seen growing in the pith up to the merismatic region of the apex. × 68.

Figs. 2 and 3. Portions of Fig. 1 more enlarged. Fig. 2 from part marked *A*, and Fig. 3 from that marked *B*. Showing longitudinal tracts of intercellular mycelium with some haustoria: at *A*. a horizontal tract is given off. × 420.

Fig. 4. Similar longitudinal section of pith at apex of spring shoot, with intercellular mycelium and haustoria. The parenchymatous cells near the mycelium are more elongated than the others, and less divided by horizontal walls. × 420.

Fig. 5. Transverse section of young pith, with transverse sections of intercellular mycelium-threads. The walls in which the mycelium-threads occur are swollen. × 420.

Fig. 6. Longitudinal section of the phloem of an infected branch of witches' broom. In the elongated cells the haustoria are not knot-like.

Fig. 7. Medullary ray in transverse section of an infected stem, with mycelium and haustoria. × 420.

Figs 8 and 9. Medullary rays in tangential section of an infected stem, with mycelium and haustoria. The walls in which the mycelium-threads occur are much swollen. × 420.

Fig. 10. Tangential section of the very young wood in the neighbourhood of the cambium. The cell-contents are plasmolytically contracted, and withdrawn from the longitudinal walls, but not from the oblique transverse walls. × 420.

Fig. 1.

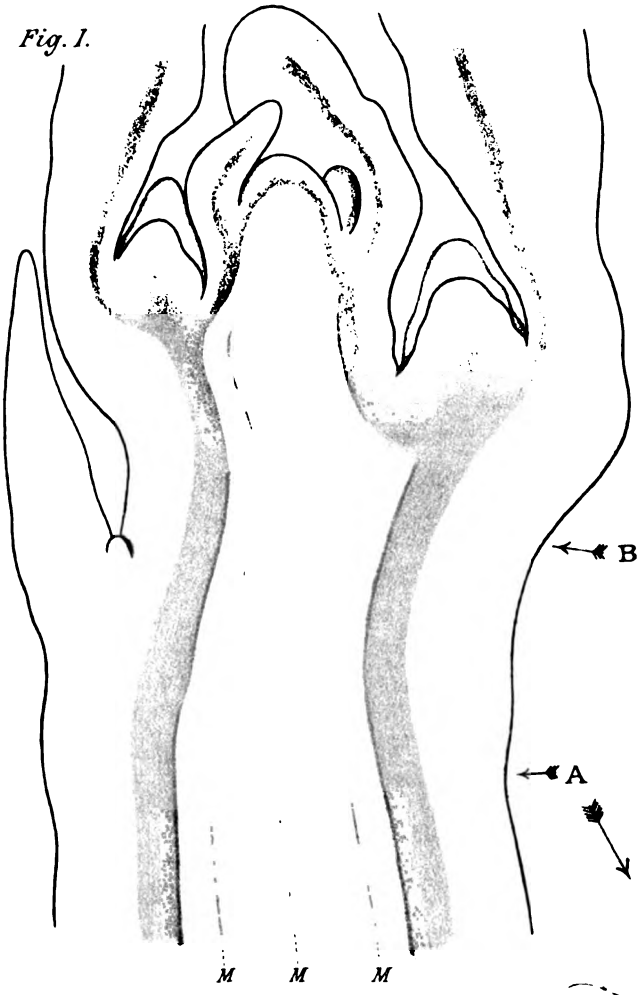


Fig. 3.

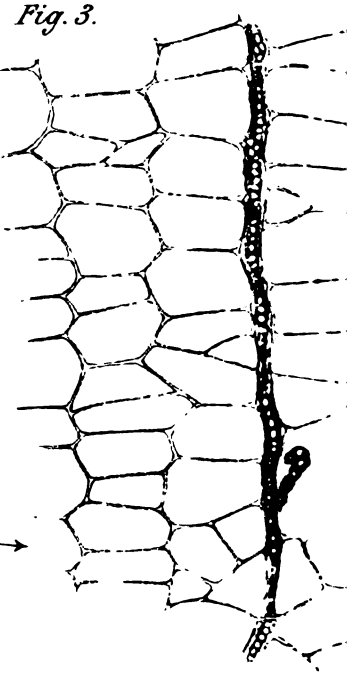


Fig. 2.

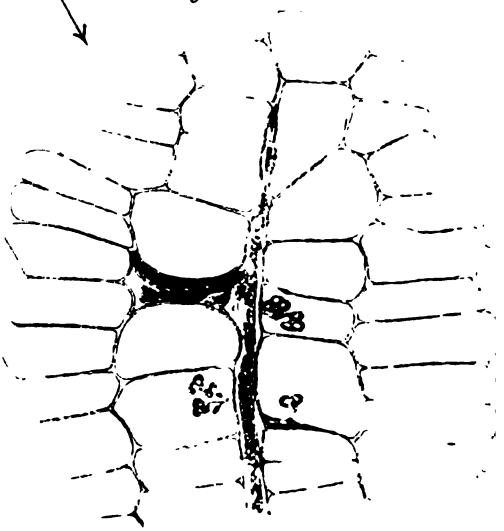
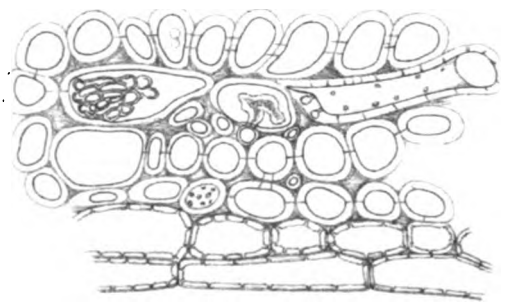


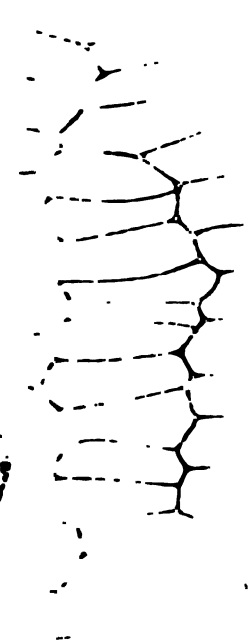
Fig. 7.



P Röseler del

MAGNUS. – MYCELIUM OF AECIDIUM GRAVEOLENS.

Fig. 4.



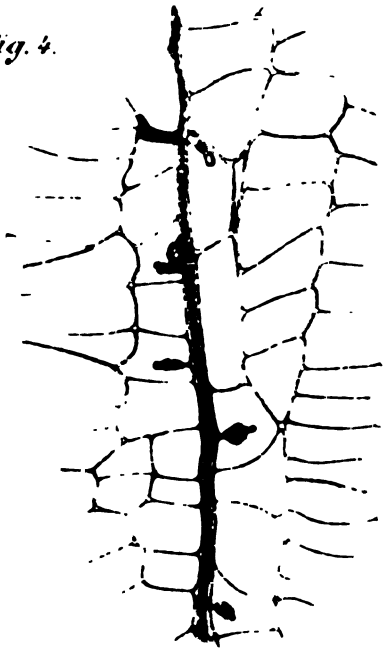


Fig. 6.

Fig. 5

Fig. 8

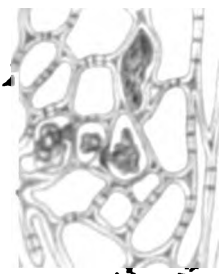


Fig. 9

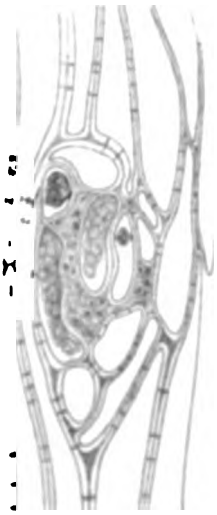


Fig. 10.

...versity Pre... at...

The Coagulation of Latex.

BY

R. H. BIFFEN,

Frank Smart Student, Gonville and Caius College, Cambridge.

WHILE engaged during the latter part of 1896 in studying the functions of latex, my attention was frequently called to its spontaneous coagulation when in contact with the air.

De Bary describes the phenomenon as follows[1]:—'As soon as latex comes in contact with the air, and still more quickly on treatment with water, alcohol, ether, or acids, coagula appear in the hitherto apparently homogeneous clear fluid itself, and independently of the aggregation of the insoluble bodies described by Mohl (Bot. Zeit. 1843, No. 33). The coagula collect together and separate with the insoluble bodies from the clear fluid. These phenomena of coagulation which appear under the action of so various agencies point especially to a complicated composition of the fluid, and deserve further investigation.'

An examination of the subject was therefore commenced with the small quantities of latex obtainable from plants grown for the purpose in the Cambridge Botanical Gardens. The results obtained were of some interest, and accordingly the experiments were continued, together with other researches

[1] De Bary, Comp. Anat. of Phanerogams and Ferns, p. 184.

[Annals of Botany, Vol. XII. No. XLVI. June, 1898.

on a larger scale, in Mexico, Brazil, and the West Indian Islands.

Rubber-yielding plants which always have laticiferous cells, were for the most part chosen on account of the ease with which large quantities of latex could be obtained, and because the various processes used in the preparation of crude rubber seemed likely to throw some light upon the subject.

A microscopic examination of any one of these latices shows that its milky appearance is due to the presence of innumerable small granules of caoutchouc, which in themselves are soft and sticky, for they readily cohere to form a small mass of rubber if the cover-glass is lightly rubbed on the slide.

Some of the processes employed to prepare this rubber may be described here.

In the preparation of Pará rubber, a thin layer of the latex of *Hevea brasiliensis* (Muell. Arg.) or other species of *Hevea*, is exposed to the action of the smoke of burning 'urucuri' nuts (*Attalea excelsa*, Mart.); coagulation is immediately brought about, resulting in the formation of a soft, curdy mass of rubber, which on drying becomes tough and elastic.

The same process is now being applied with good results to the preparation of Ceará rubber from the latex of *Manihot Glaziovii* (Muell. Arg.).

The usually accepted explanation of this is that the water contained in the latex is simply evaporated off[1]; but as the coagulation is brought about in so short a time, and moreover as there is no loss of weight on its occurrence, this is obviously incorrect.

On passing the smoke of the burning Attalea nuts through a condenser, condensation occurs and two layers of liquid are found in the receiver, one colourless and limpid, the other dark brown and oily. If these are separated by means of a pipette, or with a moistened filter paper, and analyzed, the former is found to consist mainly of acetic acid, and the latter of creosote and traces of pyridine derivatives.

[1] Ernst, Trinidad Bulletin, vol. iii. p. 235.

On adding acetic acid to the crude latex of *Hevea* coagulation occurs immediately. This process of smoking the latex may then be classed with those mentioned by De Bary under the heading of treatment with acids. As other examples, the preparation of Lagos rubber from the latex of *Ficus Vogelii* (Miq.), in which case lime-juice is added [1], and Helfer's process of adding acetic acid to the latex of *Artocarpus Chaplasha* (Roxb.) [2], may be quoted.

It is worthy of note that the latex of *Hevea brasiliensis* is in itself alkaline, and that the addition of a solution of ammonia preserves it indefinitely from spontaneous coagulation. The addition of alkalies bring about coagulation, however, in the latex of *Castilloa elastica*. In Mexico and Nicaragua, where this tree abounds, a decoction is made of the stems of the Moonflower, *Ipomoea bona-nox* (*Calonictyon speciosum* [Choisy]), and added to the latex [3]. The alkaline properties of this extract are well known to the native Indians, who frequently employ it in the manufacture of soap. The latex has an acid reaction towards litmus-paper, and the addition of acids does not cause coagulation.

Another method of clotting latex is to add an excess of common salt. This method is almost invariably applied in the case of *Hancornia speciosa* (Gomez) to produce the 'mangabeira' rubber. It is also reported to have been employed at times to coagulate the latex of species of *Hevea* and *Manihot Glaziovii* (Muell. Arg.).

Coagulation may also be brought about by boiling the latex, as, for example, in the preparation of 'balata' from *Mimusops globosa* (Gaertn.) in Venezuela and Trinidad.

There are several other methods in general use besides the few that have been quoted, and many others have been suggested from time to time [4].

[1] Kew Bulletin, 1890, Art. 142, p. 89.

[2] Watt's Dict. Economic Products of India, vol. iv. p. 343.

[3] Belt, Naturalist in Nicaragua, p. 33.

[4] For a complete account see *Le Caoutchouc et la Gutta-percha*, Seeligman, Lamy, and Falconnet, Paris 1896.

As the rubber exists in particles in the latex, it seemed possible that the centrifugal method of separation might be adopted in examining the phenomena of coagulation. A modified form of the ordinary centrifugal milk-tester was therefore designed capable of being rotated some 6,000 times per minute.

The latex was taken directly from the trees, strained through wire-gauze to remove any pieces of bark, and then, if very thick, diluted to about the consistency of thin cream. The first experiments were made with the latex of *Castilloa elastica*. After centrifugalizing for from three to four minutes, the rubber-particles completely separated as a thick, creamy, white layer, from the deep brown solution containing tannic acid in which they had been suspended. This layer was taken off, shaken with an excess of water to thoroughly wash it, and again separated. The separated particles were then shaken with water so as to form an emulsion, and alkalies were added. No coagulation now occurred, even though the mixture was allowed to stand for several days. The particles could however be brought into a solid mass by pressure, by gently heating, or by drying off the water with a porous tile.

So prepared, the rubber formed a pure white mass, without any trace of its usually characteristic smell. On exposure to the air for several days the surface gradually became brown, probably owing to oxidation.

The percentage of rubber in the latex was estimated at the same time by separating 50 c.c. The weight of the dry substance was 12·5 grammes, which, as the specific gravity of *Castilloa elastica* latex is practically 1·0, gives a yield of 25 per cent.

On treating the latex of *Hevea brasiliensis* in the same way for a slightly longer time a similar separation occurred. The same purely physical means as those employed in the case of the separated *Castilloa* rubber-particles caused them to coalesce to form a solid mass, while the addition of acetic acid and the action of the smoke of burning urucuri nuts had no effect.

The yield of rubber, estimated as before, was from 28 to 30 %. The latex of *Manihot Glaziovii* also separated readily and gave results completely parallel with those mentioned above. This latex is interesting, as it is readily clotted by churning. A soft spongy clot is formed in a few minutes containing in its meshes the greater part of the solution in which the rubber-particles were suspended. If this clot is cut into slices while still soft, and pressed between sugar-cane crushers, or in a heavy press, the bulk of the solution is extracted and a fairly pure rubber is found. On drying it does not give off the putrid smell characteristic of the ordinary Ceará 'scrap.'

Other latices can also be clotted by churning, but the process is a long one.

The latex of *Hancornia speciosa* and of *Mimusops globosa* gave similar results on centrifugalizing. In the case of the latter the pink colouring-matter which characterizes 'balata' was found to have separated as a thin layer at the bottom of the tubes.

Artocarpus incisa (Linn.) contains a very viscous latex employed by the Brazilians as a bird-lime or as a substitute for glue. When diluted and centrifugalized it separates readily, giving a creamy white layer which dries to a resinous mass somewhat resembling gutta-percha. At the ordinary temperature this is quite hard and brittle, but if the temperature is raised slightly it becomes plastic, and at the temperature of boiling water it is soft and excessively sticky. The substance is soluble in carbon bi-sulphide, and insoluble in alcohol and water.

Urostigma gamelleira (Miq.[1]) yields a similar substance of a chocolate-brown colour.

We thus see that the mere action of centrifugal force effects the separation of rubber; and from the failure of the processes usually employed, involving the use of chemical reagents, to bring about the clotting of the separated and washed rubber-particles, we must infer that no chemical change occurs in the

[1] Mart. Fl. Bras. 4. 1. 93, *Ficus doliarum* of Mart. Sys. Mat. Med. Bras. p. 88.

rubber itself, and that the cause of coagulation must be looked for in the medium in which they are suspended.

From our knowledge of the constitution of latex it is evident that the proteids are the most likely substances to cause this when treated with acids, alkalies, excess of salt, &c., and when boiled.

Unfortunately few latices have as yet been examined for their proteid constituents, chiefly on account of the difficulty of obtaining them in their natural condition in European laboratories, owing to their coagulating and undergoing decomposition during the journey from the tropics [1]. The investigations so far made prove the presence of albumin, globulin, albumose, and peptone in several rubber-yielding latices [2]. In the clear solution left after separation of the rubber-particles the xanthoproteic reaction always showed the presence of proteid matters, but under the circumstances it was impossible to identify them.

Now albumins are characterized by the coagulation of their solutions on heating, especially in the presence of dilute acids, and globulins by their ready precipitation with the salt-solution and their coagulation on heating.

Thus when the latex of *Hevea brasiliensis* is held in the smoke of the burning urucuri nuts, the albumin it contains [3] is clotted by the action of heat in the presence of dilute acetic acid.

The globulin of *Manihot Glaziovii* latex coagulates on heating when the temperature rises to 74–76° C.[4]

The acid latex of *Castilloa elastica* contains an acid albumin, which on neutralization forms a gelatinous precipitate.

These coagula on forming gather up the rubber-particles (and probably starch-grains also, in the case of starch-containing latices) in the same way as the white-of-egg gathers

[1] This does not apply to the latex of *Mimusops Globosa*, or *Hancornia speciosa*, both of which may be kept for months without undergoing any change.

[2] J. R. Green, Proc. Roy. Soc. 1886, p. 28.

[3] Faraday—see *Le Caoutchouc et la Gutta-percha*.

[4] J. R. Green, ibid.

up particles in suspension when clotted for the purpose of clearing jellies. We may even push the old analogy of blood and latex further, and compare the formation of a rubber-clot, in many cases, to the formation of a blood-clot, the rubber-particles being bound together by coagulated proteids in the same way as the blood-corpuscles are bound together by fibrin. In this case, however, we must remember that the rubber-particles, owing to their being sticky bodies unprotected by any external film, as *e.g.* the fat-particles of milk are, are capable of aggregating together of their own accord to form a solid mass.

Rubber then, as now prepared, contains among other substances proteid matters. To these must be ascribed the well-known 'fermentative change' which causes a considerable loss by converting the solid blocks of rubber into a foul-smelling spongy substance. In the Pará rubber the creosote, absorbed from the smoke of the burning nuts, acts as an antiseptic and prevents this proteid decomposition [1].

To test for the coagulated proteids is not an easy matter; continued boiling with a concentrated solution of caustic potash will however extract small quantities of alkali-albumin. 'Balata' gives good results most readily. On extraction with caustic potash a flocculent precipitate is obtained, which is readily soluble in dilute nitric acid, and is reprecipitated on the addition of alkalies. Boiling precipitates it either in acid or alkaline solutions, and it gives no precipitate with acetic acid and potassium ferro-cyanide. The proteid is thus identical with the albumose described by Green from the latex of *Mimusops globosa*.

[1] Cf. the smoking of fish &c. for preserving purposes.

BOTANICAL LABORATORY, CAMBRIDGE,
February, 1898.

The Development of the Cystocarp in Rhodymeniales:

II. Delesseriaceae.

BY

REGINALD W. PHILLIPS, M.A., B.Sc.,

Professor of Botany in the University College of North Wales, Bangor.

With Plates XV and XVI.

IN a former paper in the Annals of Botany ('97) I published the results of observations on the development of the cystocarp in certain species of the families Bonnemaisoniaceae, Rhodymeniaceae, Sphaerococcaceae, and Ceramiaceae. In still earlier papers ('95 and '96) I had already described the structure of the ceramidia of several species of Rhodomelaceae. But the cohort Rhodymeniales as constituted by Schmitz ('89) contains yet a sixth family, the Delesseriaceae; and in this paper I propose to give the results of an investigation of the structure of the cystocarp in the following species belonging to it:—

DELESSERIA SANGUINEA, Lamx.
DELESSERIA ALATA, Lamx.
DELESSERIA HYPOGLOSSUM, Lamx.
DELESSERIA RUSCIFOLIA, Lamx.
DELESSERIA SINUOSA, Lamx.
NITOPHYLLUM LACERATUM, Grev.
NITOPHYLLUM HILLIAE, Grev.

[Annals of Botany, Vol. XII. No. XLVI. June, 1898.]

DELESSERIA SANGUINEA, Lamx.

This plant is one of the most conspicuously beautiful of all the Red Seaweeds, and must have been known from early times. It was the *Fucus sanguineus* of Linnaeus (1767), and on the disintegration of that comprehensive genus became the *Delesseria sanguinea* of Lamouroux ('13). J. G. Agardh ('51) could not, however, find that its characters harmonized with those of other species of *Delesseria*, and he therefore adopted for it the generic name *Wormskioldia*, proposed by Sprengel ('27), but unlike that author, made it the single species of the genus. Later ('76), finding that this name had already been appropriated, Agardh utilized Stackhouse's ('01) generic name *Hydrolapathum*. More recent writers have assigned it to *Delesseria* or to *Hydrolapathum* as they recognized or denied its near relationship to such other typical species of *Delesseria* as the *D. Hypoglossum*, *D. alata*, and *D. ruscifolia* of Lamouroux. Kützing, by transferring such species as those last named to a genus *Hypoglossum*, and retaining the designation *Delesseria* only for *D. sanguinea* and two South Atlantic plants, has shown a certain agreement with Agardh's view of the generic distinction of the *Delesseria sanguinea* of Lamouroux from the other species of that author. Schmitz ('89), in his 'Uebersicht,' has reverted to the older arrangement of Lamouroux. This course has, as late as last year ('97), called forth a protest from Agardh, who re-asserts his belief in the generic isolation of *D. sanguinea*.

There is, moreover, a wider question at issue among algologists in connexion with this species. While Kützing has separated *D. sanguinea* from other species of *Delesseria*, he has retained it in the same family with them. Agardh ('76), however, and in this he has been followed by Hauck ('85) and others, has removed it altogether from among Delesseriaceae, and placed it among Rhodymeniaceae. This course he still defends in his latest publication ('97).

It thus appears that there is a difference of opinion, not

only upon the generic position of *D. sanguinea*, but even upon its ordinal place. I propose to recur to this question later.

The segments of the thallus of *D. sanguinea* simulate the appearance of leaves to a remarkable degree. As, however, Goodenough and Woodward (1795) long ago remarked, 'when attentively considered and compared with others, they appear to be branches growing up into, or dilated into, a thin membrane.' The rudimentary plant consists of a leaf-like lamina, attached by a holdfast. In course of time the winged portion of the thallus disappears, and further growth is provided for by proliferation from the persistent, more or less cylindrical midrib. I believe that in this species a new series of proliferations occurs every year, and that therefore the age of the plant can be accurately measured by counting the joints of the sympodium, of which the plant, from the holdfast to the still growing apex, consists. Among the first proliferations of each recurring period of growth the fertile shoots arise in great numbers (Fig. 1). Occasionally the winged portion of an old shoot may persist for some time after the proliferations of a new period have begun to grow, in which case the fertile shoots appear in two rows, right and left of the midrib, on each surface of the leaf-like shoot. These fertile shoots are at first precisely similar in appearance to the corresponding stages of the sterile shoots, but remain small in comparison to the later stages of the latter. The production of reproductive organs, whether tetrasporangia or antheridia or cystocarps, seems to drain the resources of the shoots and to dwarf their vegetative growth. Whilst the sterile shoots of one period may reach many inches or even a foot in length, the tetrasporiferous shoots are hardly more than half an inch long, and the antheridiferous and cystocarpic shoots are even less. I believe, however, that when fertilization fails, procarp-bearing shoots may, and often do, take on again the vigorous apical growth of the sterile shoots, and later become indistinguishable from them.

When, however, fertilization does take place, the female

shoots gradually become transformed into the so-called 'pedicellate' cystocarps. These usually occur in considerable numbers along the lateral margins of the midribs, and have much the appearance, when mature, of aggregates of minute berries. By a careful comparison of a series of these structures of varying degrees of maturity, the transformation of the young proliferations into the ripe cystocarps may be made out. With the aid only of such magnification as is afforded by a simple lens the following changes may be observed to occur.

The young shoot in its primary condition is about 1 mm. long, and is lanceolate in outline, its width at its widest part being about twice that at its base (Fig. 2). It is perceptibly thicker along the mid-line, as if traversed by a rudimentary midrib. The first appearance of a cystocarp is a slight swelling at the mid-line on one of the surfaces. No dorsiventrality can be detected in these proliferations, and the swelling may arise on either surface. The circular base of this swelling gradually extends to the margins of the lamina, so that the outline is changed from lanceolate to ovate (Fig. 3). Widening still more, the outline might be described as rotund, were it not that a small triangular apical region of the originally lanceolate thallus persists as such (Fig. 4). In elevation the swelling rises so that the vertical diameter is soon as great as the horizontal diameter (Figs. 5, 7), and the swelling is roughly dome-shaped. With the growth of the cystocarp there becomes apparent near the summit of the dome a pore, the margins of which, later, somewhat protrude, transforming the dome into a broad-based urn. The lamina upon which the urn is situated does not remain flat, but, with the progress of growth, becomes somewhat depressed and convex below. The cystocarp, however, never becomes globular, the one-sidedness of the swelling and the existence of the triangular apical flap rendering such a term inappropriate. With the appearance of the cystocarp in the substance of the thallus its apical growth is arrested, and the urn-shaped swelling soon occupies almost the whole of the surface. When

mature the fructification assumes a dark-red colour, from the dense 'nucleus' of highly-coloured carpospores.

It would appear, therefore, that the so-called stalked cystocarp is more accurately described as a minute flattened branch, upon one of the surfaces of which an urn-shaped cystocarp has arisen.

It is, however, with the minute structure of this cystocarp, and the details of its process of maturation, that I am chiefly concerned. In order to make the following description intelligible, it is necessary to recall the histological structure of the sterile fronds of *Delesseria sanguinea* and its congeners. This has been the subject of detailed investigations by several observers, and it appears that these species conform rigidly to the law of growth of the Floridean thallus first clearly enunciated by Schmitz ('83). That is to say, the growth is exclusively apical, no transverse division ever occurring in a segment cut off from the apical cell, and no longitudinal division passing through the organic axis of the segment. It is true that Schmitz, returning to this subject later ('92), somewhat limited the application of this law, excluding in particular from its operation the tribe Nitophylleae of the order Delesseriaceae. The tribe Delesserieae, which, standing as it does next to the excluded tribe, it may be assumed that Schmitz examined afresh, was, with the large majority of Florideae, still regarded as falling under the law of exclusive apical growth. With this view I agree, as I have seen nothing in my observations on Delesseriaceae which could be regarded as evidence of intercalary cell-division. Wille ('87) has, it is true, both described and figured intercalary cell-formation as occurring in *D. sanguinea* in the axial row of cells during the course of the development of the midrib. As, however, he makes the statement incidentally and without comment, although Schmitz's work had been published some years before, it is probable that the statement was based on a too superficial observation of the thallus. The cells shaded in Figs. 1 and 3 of Taf. I of his work, are the products of the pericentral cell lying immediately above the central

cell. By appropriate means, the much longer, somewhat attenuated axial cell may be seen lying below these cells, and it may be traced, even in the vegetative thallus, in an undivided condition for great distances behind the situation described by Wille. In the young proliferations in which the cystocarps arise, there is no difficulty in tracing the undivided cells of the axial row from base to apex.

I now proceed to describe the structure and arrangement of the procarps in these phylloid branches. For some few cells behind the apical cell, the axial cells, while they give off laterally pericentral cells, which grow out to form the lamellar wing, right and left of the mid-line, do not cut off cells parallel to the flat surface of the thallus. This, however, soon occurs, and immediately following upon the appearance of these pericentral cells above and below, is the appearance of the carpogonial branch. The pericentral cell cuts off, obliquely, right or left posteriorly, a cell, which is the first cell of a 4-celled carpogonial branch. The branch curves round right or left of the pericentral in a plane roughly parallel to the surface, and in such a way that the carpogonium itself is brought forward to a level with the apical part of the pericentral cell which bears the branch, and the trichogyne there passes out obliquely to the surface. The trichogyne is inflated at the extremity, and extends but little beyond the surface. Of the four cells of the carpogonial branch, the first two are each larger than the third and fourth, and the second is considerably larger than the first. As this cell takes up Hoffmann's blue readily, the position of the carpogonial branch can easily be determined even with a low power by its means. The third and fourth cells are small, and are with difficulty distinguishable from one another for some time after the cell-division which gives rise to the carpogonium.

Such a carpogonial branch is borne by the vertically situate pericentral cell of every joint along a considerable length of the fertile branch. They lean however to the right or left of the mid-line in regular alternation. Further, when

the corresponding pericentral cells on the opposite surface are examined, it is found that they also each give rise to a carpogonial branch which curves round the pericentral cell in the same direction as the carpogonial branch corresponding to it above. These relationships will be most readily realized by means of figures. Figs. 8 and 10 show the arrangement of the carpogonial branches as seen from the surface. Fig. 9 gives the appearance of the pairs of carpogonial branches when viewed from the side. The section is supposed to be taken a little to one side of the median line, and the difference in the depth of the shading of the alternate pairs is intended to indicate the slight difference of level. A single thallus-segment thus often gives rise to from thirty to forty procarps, the position of all of which can be distinguished in material appropriately stained, and swollen in glycerine. No procarp ever arises elsewhere than in these situations along the midrib.

It does not follow, however, that all these procarps are functional at the same time. They are produced in acropetal succession, and those whose trichogynes protrude at any time are a few in the apical region on each surface. Further back, the midrib becomes stouter by the peripheral growth of the sterile filaments derived from the vertical pair of pericentral cells. Right and left of the middle line, moreover, the lateral pair of pericentral cells and their derivatives give off cells to each surface. By this process the midrib soon becomes six or eight cells thick, and the procarps, which are at the level of the cells nearest to the axis, tend to become more and more immersed. A furrow may at first be detected on the surface on each side of the midrib, joining the points of exit of the trichogynes, where minute pit-like depressions for some time remain. It may well be that the convection of spermatia to the trichogynes is facilitated by the existence of this groove, where they might more readily lodge than on the even surface.

As I have already said, I am inclined to think that some of the procarp-bearing segments grow out into the ordinary

vegetative leaf-like shoots when fertilization fails. They resume the vigorous apical growth, and, ceasing to give rise to procarps, attain great lengths. This I infer from the fact that in young branches which are considerably longer than those in the receptive stage, I could still distinguish the remains of procarps in the basal region. Vegetative shoots certainly arise here and there in the fringe of fertile shoots. Such a reversion cannot, however, occur on a large scale; for the young procarp-bearing proliferations may be counted by scores, while the number of vegetative branches on a plant hardly reaches a dozen.

The first indication of the appearance of a cystocarp in these fertile branches is best afforded by staining. One cell of the axial row, the four pericentral cells connected with it, and the adjacent axial cells before and behind, seven cells in all, become so deeply stained by Hoffmann's blue, that this region can then be readily distinguished with the aid of even a hand-lens. I have always found that this stain is taken up with greatest avidity by those cells in which there is great metabolic activity, or a relatively large quantity of protoplasm. Thus, the apical cell, the cells of the carpogonial branch, the auxiliary cell, and carpospores and tetraspores in Florideae, all stain deeply. In the ordinary vegetative cells, it is only the chromatophores and the nucleus that stain readily, the ordinary cytoplasm being hardly tinged in glycerine-material. The deep staining of the cells referred to above indicates that one of the procarps connected with the axial cell, which is the centre of the group, has in all probability become fertilized, and that physiological changes ensue in neighbouring cells, analogous to those which occur in an ovary when the seeds are fertilized. When this stage is reached, however, it may be inferred that a considerable time has already elapsed since the attachment of the spermatium to the trichogyne. In the cases examined, the trichogyne was already so much immersed and obliterated, that it was useless to look for evidence of the presence of the spermatium.

Such deeply stained groups of cells show also a consider-

able modification of parts, though each is still capable of identification with the earlier condition already described. First, the filaments, other than the carpogonial branch, derived from the same pericentral cell with it, which, when no fertilization takes place, help to form the thickened midrib, now take on a characteristic appearance. These filaments are two in number; one, the larger, springing laterally, and the other, smaller, posteriorly (Figs. 12 and 13). In all, the tuft which these filaments constitute consists of a score or so of cells. When fertilization of a procarp takes place, these cease to grow further, although all the adjacent filaments in a similar situation take on a more active growth. The cell-walls become greatly thickened and highly refractive, and sharply contrast on this account with the rest of the tissue. The neighbouring filaments growing more vigorously, soon arch over and bury them, without however completely closing the aperture above. The gap thus left is the apical pore of the future cystocarp (Fig. 16). A small portion of the external surface is thus covered in, and may still be distinguished by the foreign substances adhering to it. Both these changes, that in the tuft of filaments which cease to grow, and that in the adjacent filaments which grow the more vigorously, indicate beyond doubt on which side of the thallus fertilization has taken place. I have never found these changes taking place on both sides the thallus, or at more than one spot on the thallus. As it is unlikely that only one procarp becomes fertilized on a branch, it is probable that the demand for nutrition consequent upon the occurrence of the first act of fertilization prevents the formation of a second cystocarp. The case is analogous with that of the ovule of *Pinus*, for example, where of many possible embryos only one normally matures.

To turn however to the carpogonial branch and the pericentral cell from which it is derived. At the stage above described it is still possible to distinguish the cells of the carpogonial branch, especially since they form a characteristic filament owing to the inequality in the size of the successive cells. The cells, however, have by this time greatly altered

in appearance. Instead of readily taking up the stain, they are now the least stained in the whole section. No longer full of dense protoplasm, they are now vacuolated and granular. It is noteworthy, however, that the outline of the carpogonium is larger than before fertilization. The pericentral cell itself, at the earliest stage which I could obtain, had already divided as in Fig. 13, cutting off a large cell towards the apex of the branch. This derived cell is much larger and more conspicuous than the pericentral cell, which is greatly reduced in size by its formation. It is from this cell, undoubtedly, that the gonimoblast-filaments afterwards arise (Fig. 14); and it is highly probable that it is this cell, and not the pericentral cell, which constitutes the auxiliary cell and is fertilized by means of an ooblastema-tube from the carpogonium. Were the pericentral cell itself the auxiliary, it might be fairly argued that it would directly give rise to many gonimoblast-filaments, which it does not. An almost precisely similar case is that of *Polysiphonia*, in the Rhodomelaceae, where a cell derived from the pericentral cell is now considered to be the auxiliary, since from it, and not from the pericentral cell, the gonimoblast-filaments arise. Other Rhodomelaceae, like *Chondria*, occur, in which the pericentral cell seems to be the auxiliary. In all Ceramiaceae, it is a cell derived from the cell bearing the carpogonial branch; and this I believe to be the case here. I did not succeed in finding any trace of the ooblastema-tube. It may be noticed, however, that the close contiguity of the carpogonium to the auxiliary cell is favourable for the process of fertilization.

Fig. 14 represents an early stage in the development of gonimoblast-filaments from the auxiliary. The early cells of these filaments have an appearance which I have repeatedly observed. They are disc-like in shape, and seem to be separated by concave walls, fitting one into another like a series of cups. They probably arise in quick succession, pushing forward into the dense mucilage derived from the decadent sterile filaments. The pressure thus produced

reacts on the pericentral cell, which is pushed back against the central cell, and thus lost sight of. This may possibly account for the statement that the pericentral cell gives rise to the carpospores.

After the stage represented in Fig. 14, I have not been able to find any vestige of the carpogonial branch. It probably atrophies and disappears. The rest of the development of the cystocarp consists chiefly in the luxuriant branching of the gonimoblast-filaments, by which the sporogenous tissue attains considerable bulk in this species. The sterile derivatives of the pericentral cell are pushed off, and may often be seen lying at the peripheral part of the fertile tissue. In the mature condition the contents of the cystocarp exhibit a lobed appearance (Fig. 10), owing probably to the partial separation from one another of the products of a few main branches. Adventitious filaments seem to arise along the larger branches comparatively late in their development (Fig. 15).

The mature cystocarp-bearing branches are considerably longer than the procarp-bearing branches. This is probably due to a general elongation of the cells already formed rather than to continued apical growth. Wille ('87) has shown that in this species there arise from the internal cells of the thallus in the older parts numerous hypha-like cells whose function he considers to be storage. These arise in the basal parts of the cystocarpic branches, and to some extent may account for the greater length.

DELESSERIA ALATA, Lamx.

This plant presents several striking differences of habit from *D. sanguinea*. While it possesses an equally well-marked midrib, the laminar portions are so reduced that the appearance is more that of a winged stem, which is the true morphological equivalence in both cases. In *D. sanguinea*, however, the proliferations of one season do not branch again

in the same season; and when the new proliferations arise in the next season, it is exclusively from the persistent midrib. In *D. alata* the plant bifurcates repeatedly in one plane by marginal growth near the apex; and as the apical growth is apparently continuous from one season to another, the plants come to consist of a dichotomously branched thallus of considerable length. In the neighbourhood of the axils between the branches, it also gives rise to dense tufts of adventitious shoots, similar to those which come off the midrib in *D. sanguinea*. It is on these structures for the most part that the reproductive organs occur; but as far as the production of tetrasporangia and cystocarps is concerned, they also occur, but less commonly, on the surface of the ultimate forkings of the ordinary thallus. Hence it would seem that *D. sanguinea* is a more highly specialized plant than *D. alata*.

The apical growth of the thallus is as pronounced in this species as in the other, and no true intercalary growth occurs throughout its structure. *D. alata* has been selected by Kny ('86) in his well-known 'Wandtafeln' for illustration of apical growth. Wille ('87), in his figures of the apex, seems to consider that cells of the axial row divide by means of 'horizontal' walls, i. e. by transverse divisions, which is not the case. The so-called 'hyphal' cells which arise from the inner cells some distance behind the apex in *D. sanguinea* occur also in this species. Wille regards their function here as that of conduction, not of storage.

The young axillary proliferations of *D. alata* serve well for the study of the development of the cystocarp, as in the same tuft there may be found varying stages of growth. The cystocarp arises on the midrib some distance behind the apex, and gradually enlarges as a papillar elevation until it can be seen in profile by means of a hand-lens. The mature cystocarp, however, never so completely transforms the appearance of the branchlet as it does in *D. sanguinea*. This is partly because the cystocarp is not so bulky, and partly because the proliferation is in the end larger than those of *D. sanguinea*.

When the cystocarp occurs on the midrib of one of the ultimate forkings of the thallus, it is still smaller in proportion to the size of the thallus. It is only in this condition that it is figured by Harvey ('51).

I have found the arrangement of the procarps to correspond closely to that already described as occurring in *D. sanguinea*. The carpogonial branches are 4-celled, and arise on the pericentral cells above and below. They lean to the right and left alternately as in *D. sanguinea* (Fig. 17). Of the four cells of the branch, the second is here too by far the largest, exceeding in bulk the other three put together. The trichogyne is inflated where it reaches the surface, and protrudes but little.

The next recognizable stage is elucidated by the same selective staining of the axial cell of the fertile joint, and of the six adjacent cells. At this stage the enlarged peripheral sterile derivatives of the pericentral cell are a conspicuous feature, although their appearance is dissimilar from the equivalent structures in *D. sanguinea*. They consist similarly of two branches, but the posterior branch consists of only two cells, and the other branch of four (Fig. 18). These cells are relatively much larger than in *D. sanguinea*, and form a loose aggregate of cells, whose pit-connexion it is not easy to follow.

The carpostome is formed by the over-arching of the surrounding vegetative filaments, though their growth seems to take place in a common mucilage and without any such invagination of the external surface as occurs in *D. sanguinea*. This is doubtless associated with the circumstance that the cystocarp does not attain the large size of that of *D. sanguinea*.

The gonimoblast-filaments arise exclusively from an anterior derivative of the pericentral cell, and are directed forward in the early stage (Figs. 18, 19). There is the same probability that this derivative of the pericentral cell, rather than the pericentral cell itself, is here also the true auxiliary; but the disorganization of the carpogonial branch at this stage

renders it difficult to find any evidence of conjugation of the carpogonium with either cell.

Delesseria Hypoglossum, Lamx.

The ordinary vegetative thallus of *D. Hypoglossum* proliferates regularly from the midrib, and there is never any such forking by marginal growth as is found in *D. alata*. The lateral veins which are so marked a character of the vegetative thallus of *D. sanguinea*, and which occur more obscurely in *D. alata*, are absent from this species. The segments which bear the reproductive organs are otherwise indistinguishable from the ordinary vegetative segments: hence it would appear that this species is a still less specialized form than *D. alata*.

The apical growth has long since been accurately described by Naegeli ('47). Owing to the great obliquity of the cell-divisions in the lateral pericentral cells, the apical region presents a beautiful appearance which does not occur in any other British species of the genus. I have found this character useful in distinguishing this species from *D. ruscifolia* with which it is sometimes confounded in herbaria.

The hyphal filaments which occur along the midrib in *D. sanguinea* and *D. alata*, and which in the older parts greatly obscure the primitive arrangement of the cells, do not seem to occur in *D. Hypoglossum*, at any rate at the corresponding stages.

The cystocarp-bearing plants occur only very rarely on the coast of Anglesey. Goodenough and Woodward (1795) contrast the east and west coast of England in this respect. According to these authors, it was only cystocarpic plants that had in their time been found on the coast of Norfolk. I have already ('96) referred to the case of *Plumaria elegans*. While I could never find female plants of this species on the coast of Anglesey or Carnarvonshire, I found them frequent at Sidmouth. Again, it is well known that *Laurencia obtusa* and *L. pinnatifida* rarely occur as cystocarpic plants in British waters. Mr. A. H. Church, who was good enough to send me cystocarpic material of *D. Hypoglossum* and other

species from Plymouth, suggests that temperature probably affects the frequency or infrequency of the female plants. Considering that, for the most part, male and female plants among Florideae are, in comparison with the tetrasporic plants, small and apparently depauperized, it may be that the cystocarpic plants are few where the conditions of temperature and illumination are favourable for vegetative development. It is, however, hazardous to generalize in the present state of our knowledge of the natural history of our marine Algae.

Not more than one cystocarp usually arises on *D. Hypoglossum* in the course of a single branch, though one cystocarp may often be found on each of the many proliferations of a branch which itself bears a cystocarp. The cystocarp is relatively small, and the branch persists after the cystocarp has discharged its spores. The procarps occur along the thallus in the same regular way that has been described for *D. sanguinea* and *D. alata*. On the female plants, at the proper season, every leaf-like branch seems equally to bear carpogonial branches throughout its course, until a cystocarp arises, when the production of procarps generally ceases (Fig. 21).

Since the midrib does not thicken to the extent that it does in the species previously described, it is possible to detect the unfertilized carpogonial branches for great distances along the thallus (Fig. 22). It is moreover possible to follow more readily the sequence of events in the young cystocarp. Fig. 22 represents a surface-view of the tuft of gonimoblast-filaments, derived from the auxiliary cell and the carpogonial branch may still be seen in an attenuated condition lying alongside the tuft. No signs of a conjugation of the carpogonium and the auxiliary can be seen at this stage; though the incapacity of the cells of the carpogonial branch to any longer absorb the blue stain, which they so readily take up at an earlier stage, suggests that the protoplasmic contents have undergone change. It is fair to add that all the cells of the carpogonial branch behave alike in this respect.

DELESSERIA RUSCIFOLIA, Lamx.

D. ruscifolia is undoubtedly closely allied to *D. Hypoglossum*, with which indeed it seems to have been confounded until Turner ('02) pointed out the distinguishing characters. In its dark-red colour *D. ruscifolia* resembles *D. sanguinea* rather than *D. Hypoglossum*. Its segments are oval or oblong rather than lanceolate, as in *D. Hypoglossum*; a lateral venation is obscurely traceable also in *D. ruscifolia*, and the cells are much smaller than are those of *D. Hypoglossum*. There is also a marked difference in the shape of the cystocarp, which in *D. Hypoglossum* is somewhat flattened, but in *D. ruscifolia* has an elongated neck, and a carpostome with everted rim. As in *D. Hypoglossum*, each branch of the female plant usually bears a cystocarp, though occasionally, as in Harvey's figure ('51), two may occur on the same midrib.

The procarps occur on the midrib only, but the arrangement is by no means so regular as in the three preceding species. Occasionally no procarp will occur on an axial cell, or two successive joint-cells bear procarps inclined towards the same side, or a procarp will occur on one surface but not at the corresponding situation on the opposite surface. A more important deviation is the rare occurrence of two carpogonial branches arising, one on the right, the other on the left of the same pericentral cell. The cells of the carpogonial branch are also more uniform in this species, though in this respect it resembles *D. Hypoglossum*.

The condition of the young cystocarp figured in Fig. 20 may often be found. It is probable that it represents a pause in the sequence of events between the fertilization of the trichogyne and the production of the gonimoblast-filaments. If so, this lends additional support to the idea that the anterior cell cut off from the pericentral cell is the auxiliary cell, as the halt may be accounted for by the fact that at this stage the conjugation of the carpogonium with the auxiliary cell would occur. As in the other species, it is from this anterior cell alone that the gonimoblast-filaments arise.

DELESSERIA SINUOSA, Lamx.

This plant presents so great a general similarity in appearance to *D. sanguinea*, that the collector would readily acquiesce in its inclusion in the same genus with it. As *D. sanguinea* has been appropriately called the 'Dock-leaved' *Delesseria*, so might this plant be called the 'Oak-leaved' *Delesseria*. In the remarkable similation of the veined appearance of the leaf of Flowering-plants these two species stand out conspicuously among British Seaweeds.

Kützing ('49), however, constituted for *D. sinuosa* the separate monotypic genus *Phycodrys*, and although Schmitz in his list of Floridean genera ('89) included the species in *Delesseria*, he seems later ('92) to have contemplated the possibility of its restoration to the position assigned to it by Kützing.

A brief description of the macroscopic characters will explain this disinclination to include the species in the genus *Delesseria*. Its branches are traversed by a midrib from which diverge veins into the substance of the distended lamina. This midrib persists when the winged portion disappears, and gives rise by proliferation to the new phylloid branches. More commonly, however, a thallus-segment forks by the more vigorous growth of one of the lobes. Even when a lobe does not develop so as to form a distinct segment, its vein may become a strong secondary rib, from which proliferations may arise as they do from the midrib. Again, *D. sinuosa* produces its tetrasporangia in marginal stichidia, like those of some species of *Nitophyllum*, and not along the sides of the midrib of the vegetative branches, or on special proliferating branches as in species of *Delesseria*. Further, the cystocarps are scattered in considerable numbers over the marginal region of the thallus-segment away from the midrib, while in *Delesseria* they usually occur one for each segment, and on the midrib. It is true that in the end the cystocarp of *D. sinuosa* is found to be seated on a prominent

vein, as shown in Harvey's figure, but this vein arises only after the establishment of the cystocarp.

When the manner of growth of the thallus comes to be considered, *D. sinuosa* presents a marked contrast to the species already dealt with. In these there is present at the geometrical apex a single conspicuous cell, which, by its repeated transverse divisions, gives rise to the axial row, the cells of which never divide transversely again, and from which later cells are cut off longitudinally by divisions which do not pass through the organic axis. The pericentral cells thus cut off repeat, in a modified form, the behaviour of the apical cell, and thus the thallus arises. Were it possible to isolate the pericentral cells with their respective products, the whole thallus would resolve itself into a system of branched filaments like a *Callithamnion*. The growth is apical, in the sense that multiplication of cells takes place at innumerable apices, of which the most important coincides with the geometrical apex, the others lying at the margin and surface, or imbedded in the substance of the thallus. This method of growth Schmitz at first regarded as characteristic of all the Red Seaweeds, exclusive of the Bangiaceae. Naegeli and Schwendener ('67), in their work on the Microscope, had already selected *D. sinuosa* and *Nitophyllum laceratum* as typical cases of growth by intercalation, which they illustrated by figures. Returning to this subject in his later writings, Schmitz ('92) conceded the whole tribe Nitophylleae (including *D. sinuosa*) as affording evidence in the structure of the thallus, sooner or later, of intercalary growth. He demurs, however, to the figures of Naegeli and Schwendener, which, he said, left much to be desired. He seemed still to deny that the growth in thickness of the thallus of *Nitophyllum* is ever due to intercalation, and in particular he refused to acquiesce in Johnson's ('92) suggestion that the callosities of *Nitophyllum versicolor* afforded an instance of growth by intercalation. With regard to the growth in thickness of the thallus in the neighbourhood of the cystocarps, in both *Nitophyllum* and *D. sinuosa*, my own observations convince

me that it is effected in precisely the way in which it takes place in other families of Florideae, that is to say, by exclusively apical growth. As to the growth in area, where, by inference, it is to be concluded that Schmitz believed growth by intercalation to take place, I have seen no evidence of this either, but do not claim to have given, as yet, adequate attention to the phenomena figured by Naegeli and Schwendener.

From all this, however, it is manifest that in the way in which the thallus of *D. sinuosa* arises, it is more akin to *Nitophyllum* than to the species of *Delesseria* already described.

When the arrangement of the procarps in *D. sinuosa* comes to be considered, the divergence from *Delesseria* is equally striking. They are found to be distributed in great numbers, without any regularity, in the marginal portions of the thallus. The ultimate ramifications of the veins are obscure lines, traceable only with the aid of the microscope, where a row of axial cells gives off a pericentral cell above and below. Between these veins the thallus is only one cell thick. It does not appear that the procarps in their inception are related in any way to the veins, although a strong vein always arises in connexion with a fertilized procarp. When the procarps arise, an axial cell cuts off a pericentral cell above and below, and from each pericentral cell there springs a 4-celled carpogonial branch, which curves in a characteristic manner before the trichogyne emerges slightly at the corresponding surface (Fig. 29). By the time the carpogonial branches are formed, the pericentral cells from which they originate divide again and give off externally other cells, and a minute swelling in the thallus is the consequence. The trichogynes emerge on the slopes of this swelling. When fertilization fails there is no further development, and great numbers of such unfertilized procarps may be found among the few which are fertilized. The first indication of the development of a procarp into a cystocarp is afforded by the staining properties of the central cell concerned. This extends gradually to the neighbouring axial cells. At this time too

the whole area round the spot where the fertilized procarp lies increases in thickness by the cutting off of pericentral cells, in which divisions occur parallel to the surface, giving rise to vertical rows of cells. The peripheral cells, derived from the pericentral cell which bears the fertilized procarp, do not take part in this vigorous growth, but remain in number as at the moment of fertilization. They however enlarge considerably, and assume a characteristic appearance, which is the first indication as to which procarp has been fertilized. The rows of cells surrounding these sterile derivatives arch over them, leaving a central depression which is the carpostome. Pressed by the convergence of the adjacent filaments, the sterile cells, which are five in number, become pyriform, with their pointed ends outwards, and their walls become at the same time thickened and highly refractive. Fig. 27, which represents this stage in *Nitophyllum Hilliae*, might also serve for *D. sinuosa*.

The pericentral cell cuts off a segment, which is the auxiliary cell, and from which later the gonimoblast-filaments arise. The sterile cells are then pushed off, and eventually disappear, supplying in their decadence a copious mucilage.

As will be shown, in these particulars *D. sinuosa* more nearly resembles *Nitophyllum* than the typical species of *Delesseria*. The systematic position of the species will be discussed later.

NITOPHYLLUM LACERATUM, Grev.

Nitophyllum laceratum may be regarded as the typical species for the genus as established by Greville. There is no percurrent midrib, and the cystocarps are embedded in the substance of the thallus. As it possesses, however, a distinct anastomosing venation in its older parts, and a more obscure venation throughout, it was placed by Kützing ('49) with other species like it in these respects in a genus *Cryptopleura*, an arrangement, however, which has not found acceptance.

The procarps are scattered along the margin of the thallus. Fig. 25 represents a procarp in surface-view; its similarity to that of *D. sinuosa*, as shown in Fig. 29, will be apparent at once. Fig. 24 represents a section through the thallus at a point where the pair of procarps arises.

In the later stages the sterile cells derived from the pericentral form a compact group similar to that shown in Fig. 27.

NITOPHYLLUM HILLIAE, Grev.

Nitophyllum Hilliae is the largest and firmest of all the British species of *Nitophyllum*. The thallus is for the most part more than one cell thick. Like *N. laceratum*, it has the obscure venation of Kützing's genus *Cryptopleura*. I am indebted to Mr. A. H. Church for fine specimens of this plant from Plymouth.

The procarps are scattered over the thallus as in *N. laceratum* and *D. sinuosa*. On closer examination they differ in one important particular from both these species. While each pericentral cell in *D. sinuosa* and *N. laceratum* gives rise to a single carpogonial branch, in *N. Hilliae* I have found that each pericentral cell very regularly gives rise to two such branches. These curve in a crescent on opposite sides of the pericentral cell, and the trichogynes emerge on opposite declivities of the papillar elevation which marks the position of the procarps. As two carpogonial branches emerge on each surface, the swelling marks the site of four carpogonial branches. In this respect *N. Hilliae* is alone among the Delesseriaceae here described, though isolated instances of the same phenomenon occur in *D. ruscifolia*.

In the genus *Ceramium* two carpogonial branches also arise regularly from one cell.

Fig. 26 represents a surface view of a pair of carpogonial branches. Fig. 27 represents an early stage of a cystocarp. The group of pyriform sterile cells, which belong to two filaments, have already been referred to. The auxiliary cell has been formed, but has not yet given rise to gonimoblast-

filaments. The two unfertilized carpogonial branches of the opposite side of the axis can still be traced. Fig. 28 is an enlarged view of the essential parts of Fig. 27.

When the cystocarp has matured, the papillar outgrowth becomes tuberculated in *N. Hilliae*, owing to uneven growth of the vertical filaments.

I desire now to discuss the mutual relationships of the seven species, the structure of whose cystocarps has been described, and then to consider the affinities of the family to which they belong.

To consider first the four species, *D. sanguinea*, *D. alata*, *D. Hypoglossum*, and *D. ruscifolia*. It is clear that the two plants most closely related are the two last. The leaf-like branches are equally vegetative and reproductive in both, and the branching is exclusively adventitious. In *D. alata*, while the ordinary forkings may give rise to the reproductive organs, there is a marked tendency to produce them exclusively on somewhat specialized adventitious branches. What is only a tendency in *D. alata* has become a fixed condition in *D. sanguinea*. The four species thus form a natural series of which *D. ruscifolia* is perhaps the least specialized, and *D. sanguinea* certainly the most specialized. As has been shown, the structure and the arrangement of the procarps and the development of the cystocarp in *D. sanguinea* present so many features in common with the other three, that there can be no doubt about its inclusion in the same family with them. On account of considerations arising chiefly from a study of the tetrasporiferous segments, Agardh ('76) placed the species as *Hydrolapathum sanguineum* in the family Rhodymeniaceae. This decision he has recently ('97) discussed in some detail and confirmed. He regards the stichidia or tetrasporiferous branches as unlike those of any other Florideae, finds the closest analogy in the origin and

arrangement of the spores in the genus *Chylocladia*, and is convinced from all the characters of the wide separation of *Hydrolapathum* from *Delesseria*. On the other hand, it is now clear that in the young condition of the procarp-bearing segment, in the remarkable arrangement of the procarps along the midrib in this segment, in the structure of the carpogonial branch, as well as in the general course of development of the cystocarp, there is the closest agreement between this species and the typical species of *Delesseria*. And as, from all analogy, the sexual reproductive structure affords the safer criterion in the search for affinities, the similarities here disclosed must be taken to outweigh any dissimilarities appearing in the origin and arrangement of the tetrasporangia. On these grounds, therefore, I cannot but think that Schmitz's contention that *Hydrolapathum sanguineum* should be restored to the Delesseriaceae, and even to the genus *Delesseria*, is fully justified.

D. sinuosa has had a similarly uncertain position. Schmitz ('92) was latterly inclined to divide the Delesseriaceae into two tribes, the Delesserieae and Nitophylleae, on account of differences in the mode of growth of the thallus. But this division would involve the inclusion of *D. sinuosa* among the Nitophylleae, and he therefore suggested the adoption of Kutzing's genus *Phycodrys* for its reception.

This is a course which receives strong support from the study of the development of its cystocarp. In the true Delesserieae the procarps are borne in pairs along the midribs, whereas in *D. sinuosa* they are scattered as in *Nitophyllum*. In the more compact texture of the thallus, moreover, it resembles *Nitophyllum*. In the existence of a well-marked midrib with diverging veins it resembles species of *Delesseria*. These characters seem to mark for it a position intermediate between *Nitophyllum* and *Delesseria*, and the adoption of Kutzing's proposal would meet the case.

With regard to the two species of *Nitophyllum*, *N. laceratum* comes nearest to *D. sinuosa*. It seems to be premature to suggest the occurrence of two carpogonial branches on each

pericentral cell in *N. Hilliae* as a ground for the generic separation of this species from *Nitophyllum*, though, from all analogy, it would seem to indicate a deep-seated difference.

Turning now to the diagnosis of the family Delesseriaceae as given by Schmitz and Hauptfleisch ('97) in Engler and Prantl's 'Pflanzenfamilien,' it would seem to require modification in several particulars.

1. The carpogonial branches are described as 3- or 4-celled. In all the species I examined the carpogonial branch was invariably 4-celled. I never found the number to vary from four cells in the family Rhodomelaceae either. In a recent paper on *Grinnellia americana*, Harv., a monotypic genus of Delesseriaceae, which seems to stand near to *Delesseria*, Brannon ('97), has, it is true, described the carpogonial branch as 3-celled, but as he has also stated that it arises directly from a central cell, and not from a pericentral cell, it is just possible that he has missed the real pit-connexion of these cells. Otherwise, *Grinnellia* differs from all known Delesseriaceae in the origin of the carpogonial branch, as well as from those here described in the number of the cells which constitute it.

2. The carpogonial branches are said to arise singly on an inner cell of the cortex. This does not now cover the case of *N. Hilliae*, where they regularly arise in pairs, nor exceptional cases of *D. ruscifolia*, where the same thing occurs.

3. The external pericarpial wall is described as formed by a tearing away of the cortical filaments from the middle layer of the thallus. While such a tearing may occur in certain species of *Nitophyllum* with somewhat flattened cystocarps, it is certainly not general. In *Delesseria*, the filaments surrounding the cystocarpic cavity become strongly curved, being pressed back at first by the copious mucilage derived from the walls of the sterile derivatives of the pericentral cell, and later by the tuft of spore-producing gonimoblast-filaments. Under this pressure, the cells of the filaments elongate, so as to resemble rows of cylindrical cells, yet the correlation of part to part in the course of growth is

so gradual, that no rupture of their continuity can be detected.

4. The tearing away of the pericarp is further said to be commonly omitted immediately above the auxiliary cell, where a strand of filaments remains connecting that cell with the pore of the cystocarp above. The strand of cells referred to is doubtless the group of sterile derivatives of the pericentral cell so often described in the foregoing accounts, and if so, it is not a strand of cells but a bushy tuft in *D. sanguinea*, reaching only a part of the distance to the pore, and pushed aside when the gonimoblast-filaments subsequently arise. In the other species of *Delesseria* the cells are fewer in number, and lie loosely imbedded in a mucilage above the auxiliary cell, still less resembling a strand of cells connecting it with the pore. In *D. sinuosa* and the species of *Nitophyllum* the cells are more compact, and the gonimoblast-filaments grow round and over them, justifying the description 'nabelförmig' to this stage in the appearance of the cystocarp. In all cases the pore is the gap left above these cells by the over-arching converging filaments. It is not accurate to describe the sterile group as in any way attached to the pericarp at the pore.

5. As to the formation of a second chamber below, separated from the spore-containing cavity by the middle layer as a kind of diaphragm, a condition figured by Schmitz and Hauptfleisch for *N. punctatum*, I have not been able to find it in the species examined. In the maturer stages of the growth of the cystocarp, the site of the auxiliary cell is the apex of a papilla projecting into the cavity, and while the luxuriant gonimoblast-filaments depress the base of the cavity round about it, they do not, as far as I have been able to see, enter a second cavity on the opposite side of the middle layer.

6. A general fusion of the auxiliary cell with neighbouring cells is described as taking place at the 'placenta.' While such a confluence apparently occurs in some species of *Nitophyllum*, and is very general in Florideae, it is strikingly

absent in the genus *Delesseria*. Brannon ('97) found moreover that no such fusion occurs in *Grinnellia*.

7. The pericentral cell is described as playing the part of the auxiliary. I have already given reasons for believing that the auxiliary is an anterior cell cut off from the pericentral cell. An auxiliary cell so derived occurs in most Rhodomelaceae and in all Ceramiaceae.

An interesting feature in the development of the cystocarp in the species here under consideration is the fact that, when the cells adjacent to the central cell in a fertilized procarp become charged with nutriment prior to the formation of the gonimoblast-filaments, they also become multinucleate, as many as eight or ten nuclei at times occurring in one cell. I have found that elsewhere in the thallus of *D. sanguinea* the greatly elongated axial cells contain more than one nucleus.

With regard, finally, to the systematic position of the Delesseriaceae, I have no hesitation, on the ground of the remarkable correspondence in the process of development of the cystocarp, in placing them close to the Rhodomelaceae. There is the same invariably 4-celled carpogonial branch, the auxiliary cell is derived anteriorly from the pericentral cell, and there are always found, in the early cystocarp, two sterile filaments which degenerate later into mucilage. Indeed, the observation of the way in which these sterile filaments arise in Delesseriaceae affords a clue as to their origin in Rhodomelaceae which would otherwise be wanting. In Rhodomelaceae they would seem to be vestigial structures, and the cylindrical Rhodomelaceae would seem to have been derived from forms with a flattened thallus like Delesseriaceae. The two families form one alliance; the simplest forms being represented by *Nitophyllum*, and the most complex by the polysiphonous Rhodomelaceae.

BIBLIOGRAPHY.

1. AGARDH, '51: Sp. Gen. et Ord. Algarum: Vol. ii. pars 1; J. G. Agardh. Lundae, 1851.
2. AGARDH, '76: Sp. Gen. et Ord. Algarum; Vol. iii, Epicrisis; J. G. Agardh. Lipsiae, 1876.
3. AGARDH, '97: Analecta Algologica; Continuatio iv; J. G. Agardh. Lundae, 1897.
4. BRANNON, '97: The Structure and Development of *Grinnellia americana*, Harv.; M. A. Brannon; Annals of Botany, vol. xi. Oxford, 1897.
5. GOODENOUGH AND WOODWARD, 1795: Observations on British Fuci; S. Goodenough and T. J. Woodward; Trans. Linn. Soc., vol. iii. London, 1795.
6. HARVEY, '51: Phycologia Britannica; W. H. Harvey. London, 1845–51.
7. JOHNSON, '92: Callosities on *Nitophyllum versicolor*; T. Johnson; Proc. of the R. Dublin Soc., N. S., vol. vii. Dublin, 1892.
8. KNY, '86: Botanische Wandtafeln; L. Kny; Tafel 77. Berlin, 1886.
9. KÜTZING, '49: Species Algarum; F. T. Kützing. Lipsiae, 1849.
10. LAMOUROUX, '13: Essai sur les genres de la Famille Thallassiophytes non-articulées. J. V. F. Lamouroux; Mus. Hist. Nat. Paris, 1813.
11. LINNAEUS, 1767: Mantissa Plantarum; C. Linnaeus; Holmiae, 1767.
12. NAEGELI, '45: Wachsthumsgeschichte von *Delesseria Hypoglossum*; Carl Naegeli; Schleiden und Naegeli's Zeitschrift für wissensch. Botanik, Heft 2. Zürich, 1845.
13. NAEGELI UND SCHWENDENER, '67: Das Mikroskop; C. Naegeli und S. Schwendener. Leipzig, 1867.
14. PHILLIPS, '95: On the Development of the Cystocarp in Rhodomelaceae; R. W. Phillips; Annals of Botany, vol. ix. Oxford, 1895.
15. PHILLIPS, '96: On the Development of the Cystocarp in Rhodomelaceae (II). R. W. Phillips; Annals of Botany, vol. x. Oxford, 1896.
16. PHILLIPS, '97: On the Development of the Cystocarp in Rhodymeniales; R. W. Phillips; Annals of Botany, vol. xi. Oxford, 1897.
17. SCHMITZ, '83: Untersuchungen über die Befruchtung der Florideen; Fr. Schmitz; Sitzungsber. der Berl. Akad. der Wissensch. Berlin, 1883.
18. SCHMITZ, '89: Systematische Uebersicht der bisher bekannten Gattungen der Florideen. Fr. Schmitz; Flora, Heft 5. Marburg, 1889.
19. SCHMITZ, '92: Kleinere Beiträge zur Kenntniss der Florideen, I, II; Fr. Schmitz; La Nuova Notarisia, Series III. Padova, 1892.
20. SCHMITZ UND HAUPTFLEISCH, '97: Delesseriaceae; Fr. Schmitz und P. Hauptfleisch: Die Natürlichen Pflanzenfamilien; A. Engler und K. Prantl; Lief. 149–150. Leipzig, 1897.
21. SPRENGEL, '27: Linnaei Systema Vegetabilium, edit. xvi curante C. Sprengel. Göttingen, 1827.
22. STACKHOUSE, '01: Nereis Britannica; J. Stackhouse. Bathoniae, 1801.
23. TURNER, '02: Description of four new species of Fucus; Dawson Turner; Trans. Linn. Soc., vol. vi. London, 1802.
24. WILLE, '87: Beiträge zur Entwickelungsgesch. der physiol. Gewebesysteme bei einigen Florideen; N. Wille. Halle, 1887.

EXPLANATION OF FIGURES IN PLATES XV AND XVI.

Illustrating Prof. Phillips' paper on the Development of the Cystocarp in Rhodymeniales.

The figures are to be regarded as of a semi-diagrammatic character. They were sketched for the most part by means of the camera lucida, but cells lying at different levels have often been figured together, and cells are sometimes left out for the sake of clearness. The cells hatched by means of oblique lines are in all cases those of the carpogonial branch, those hatched by means of horizontal lines in Figs. 20 and 25 are the central cell and its adjacent readily stained cells. The cells with red outlines are those of the sterile filaments derived from the pericentral cell of a fertilized procarp. In Figs. 21, 27, 28, 30, 31, and 32, the red colour is also employed for these cells, although the procarps are not yet fertilized. The auxiliary cell and its derivatives are shown with somewhat thickened outlines. In the figures disposed horizontally, the same orientation of parts is preserved throughout, that is to say, the anterior part of the thallus is to the right.

Abbreviations: *aux. c.*, auxiliary cell; *c. c.*, central cell; *carp.*, carpogonium; *carp. br.*, carpogonial branch; *cpst.*, carpostome; *gonbl.*, gonimoblast-filament; *in. st. br.*, inferior sterile branch; *l. st. br.*, lateral sterile branch; *peric. c.*, pericentral cell; *tr.*, trichogyne.

PLATE XV. Figs. 1–16.

Delesseria sanguinea, Lamx.

Fig. 1. An old midrib, giving rise to three sterile branches, and numerous cystocarp-bearing branches in various stages of development. Natural size.

Figs. 2, 3, 4. Views of the flat surface of a thallus-segment upon which a cystocarp is developing. × 15.

Figs. 5 and 7. Lateral or profile views of two stages in the development of a cystocarp upon such a thallus-segment. × 15.

Fig. 8. Front view of a procarp-bearing branch, showing the row of axial cells, the pericentral cells derived from them, and the carpogonial branches turned alternately to the right and left. × 75.

Fig. 9. Lateral view of a procarp-bearing branch at the same stage. The axial cells are shown, and the paired pericentral cells of the upper and lower surface, each bearing a carpogonial branch emerging at the corresponding surface. × 75.

Fig. 10. Mature cystocarp-bearing segment, showing the apical flap remaining, and the lobing of the contents of the cystocarp. × 10.

Fig. 11. Magnified view of a segment at the same stage, and of the same view as in Fig. 8. The eighth segment behind the apical cell has an immature procarp, afterwards the carpogonial branches are fully developed. × 600.

Fig. 12. A single procarp (unfertilized) showing the relation of the sterile filaments (red) to the carpogonial branch. × 900.

Fig. 13. A single procarp at a stage subsequent to fertilization. The carpogonial branch is still present but by no means so conspicuous relatively as in the figure. The auxiliary cell has been cut off from the anterior part of the pericentral cell. The sterile filaments begin to undergo mucilaginous degeneration. × 500.

Fig. 14. Median vertical section of a segment about the stage shown in Fig. 7. The carpogonial branch is still visible, but only with difficulty traceable in a section. The auxiliary cell has developed a minute tuft of gonimoblast-filaments. The section is drawn through the carpostome, and the invaginated portion of the external surface is shown below. × 300.

Fig. 15. A strong gonimoblast-filament giving rise to an apparently adventitious lateral branch. × 300.

Fig. 16. Lateral view of the apical region of a procarp-bearing segment. Magnification inferior to that of Fig. 11. × 350.

PLATE XVI.

Delesseria alata, Lamx. Figs. 17, 18, 19.

Fig. 17. Vertical section through a procarp-bearing segment close behind the apex. The pair of procarps corresponding to every axial cell is shown. The first and third pair of carpogonial branches are on the hither side of their pericentral cells, and the second on the farther side. × 600.

Fig. 18. A fertilized procarp in vertical section. The auxiliary cell has already divided once. The carpogonial branch is not shown. × 400.

Fig. 19. A stage later. The auxiliary has given rise to a tuft of filaments by repeated branching. × 400.

Delesseria ruscifolia, Lamx. Fig. 20.

Fig. 20. Surface view of a fertilized procarp. The cells hatched by horizontal lines are three cells of the axial row and two lateral pericentral cells. These five cells stain readily. The carpogonial branch is distinctly visible. The pericentral cell has divided off (anteriorly) an auxiliary cell. The sterile derivatives of the pericentral cell coloured red. The other cells are superficial. × 750.

Delesseria Hypoglossum, Lamx. Figs. 21, 22, 23.

Fig. 21. Two cells of the axial row close to the apex with its derivative pericentral cells. From each of these are derived sterile derivatives and a carpogonial branch. × 1200.

Fig. 22. Three axial cells far behind the apex. The carpogonial branches immersed in the tissue of the midrib and atrophying. × 300.

Fig. 23. A view from the surface, corresponding to that shown in Fig. 20, for *D. ruscifolia*. The auxiliary has grown out into gonimoblast-filaments. The sterile cells are omitted. × 750.

Nitophyllum laceratum, Grev. Figs. 24, 25.

Fig. 24. Vertical section of the thallus, showing a pair of carpogonial branches, arising from the pericentral cells of one axial cell. × 750.

Fig. 25. Surface view of a procarp. The axial cell is not shown. × 750.

Nitophyllum Hilliae, Grev. Figs. 26, 27, 28.

Fig. 26. An axial cell with its pericentral cell. Surface view. Two carpogonial branches arise from the one pericentral cell. The sterile derivatives are not shown.

Fig. 27. Vertical section of a stage later, when the auxiliary cell has been cut off. The sterile filaments are a group of cells with the pointed apices directed towards the carpostome. The carpogonial branch may still be traced. Two others on the opposite side the axis are faintly traceable. × 180.

Fig. 28. Enlarged view of the central region of Fig. 27. × 500.

Fig. 29. *Delesseria sinuosa*, Lamx. Surface view of a procarp, to show its similarity to *N. laceratum* (Fig. 26).

Fig. 30. *Rhodomela subfusca.*—Products of the pericentral cell at the stage of fertilization diagrammatically represented.

Fig. 31. Similar diagram for *D. alata, D. ruscifolia, D. Hypoglossum, D. sinuosa, N. laceratum, N. Hilliae.*

Fig. 32. Similar diagram for *D. sanguinea*, to which *Dasya coccinea* approximates.

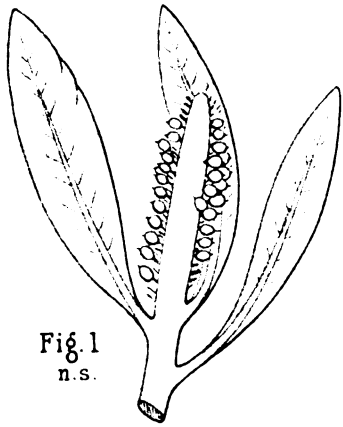


Fig. 1
n.s.

Fig. 2
x 15

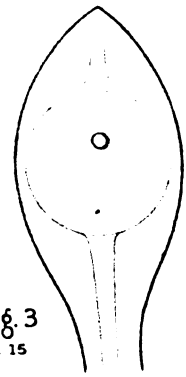


Fig. 3
x 15

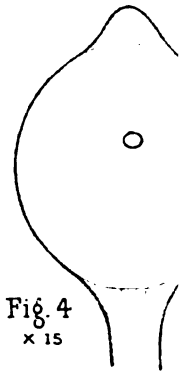


Fig. 4
x 15

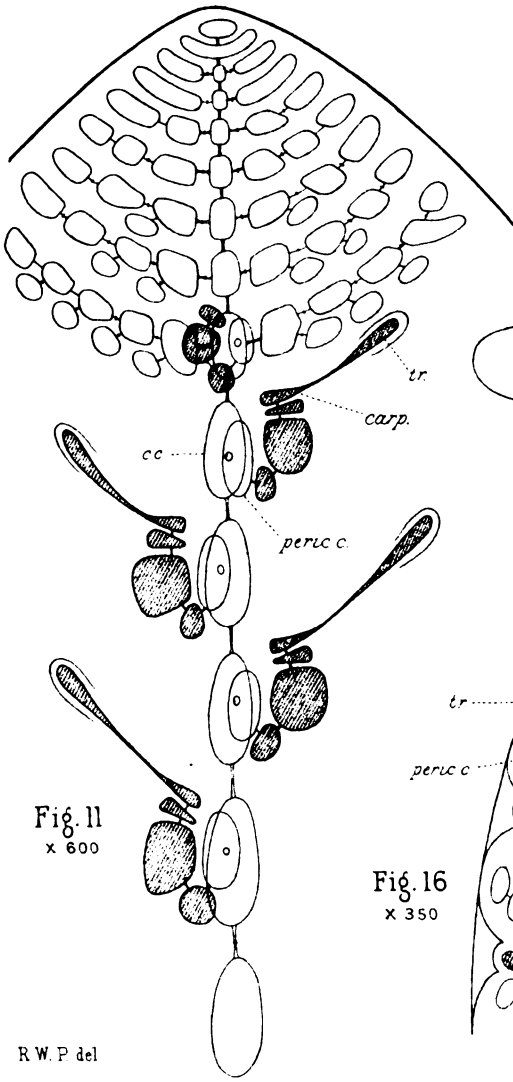
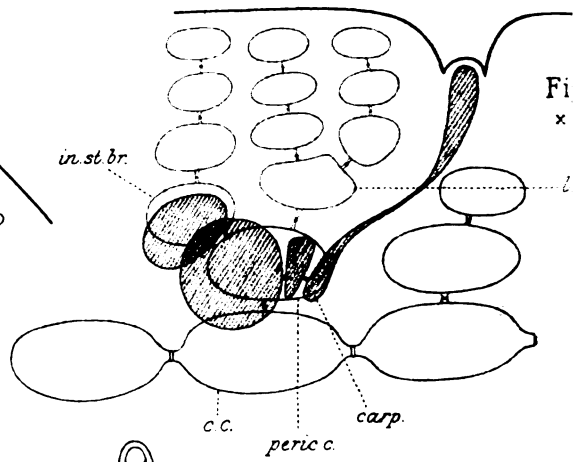


Fig. 11
x 600



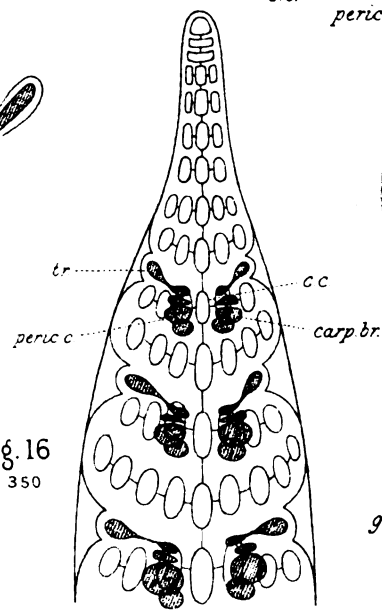


Fig. 16
x 350

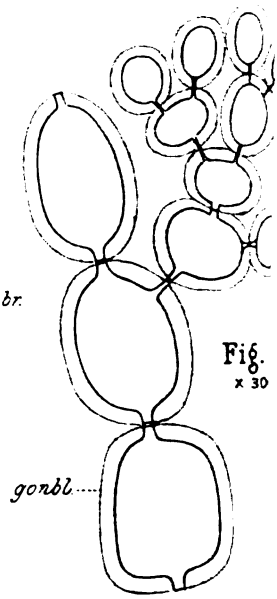


Fig.
x 30

R W. P del

PHILLIPS.— CYSTOCARP IN RHODYMENIALES, II.

Fig. 5
x 15

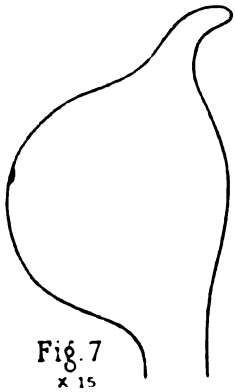


Fig. 7
x 15

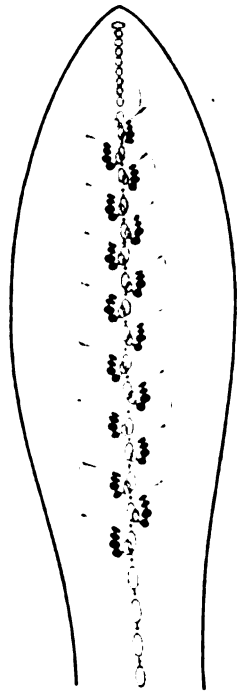


Fig. 8
x 75

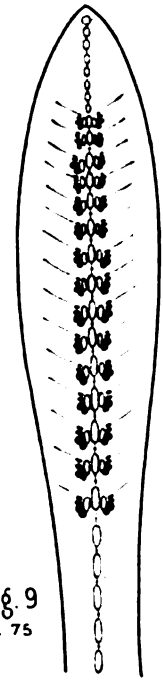
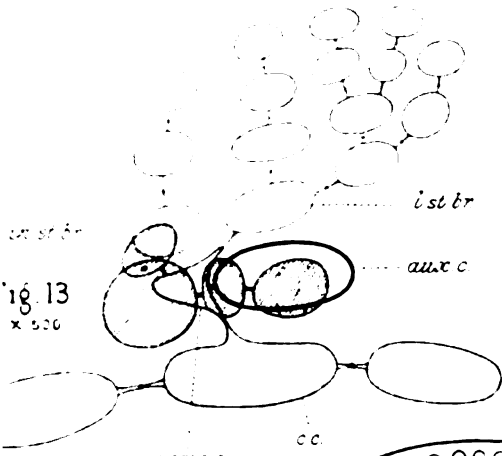


Fig. 9
x 75



ig. 13
x 300

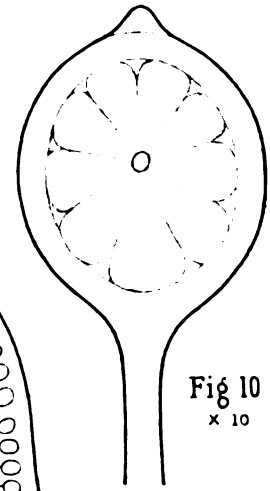


Fig 10
x 10

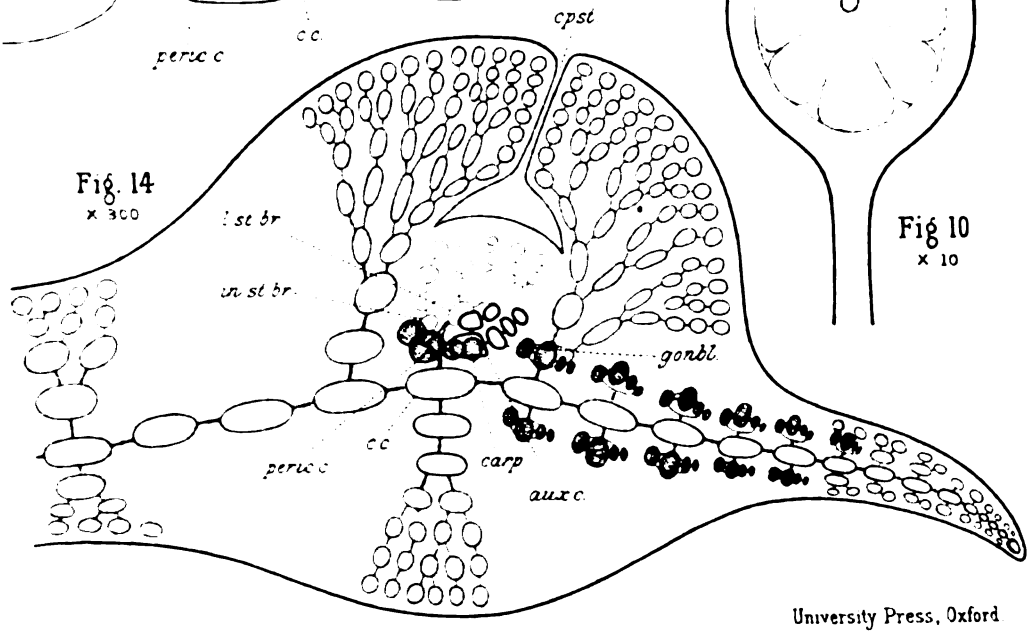


Fig. 14
x 300

University Press, Oxford

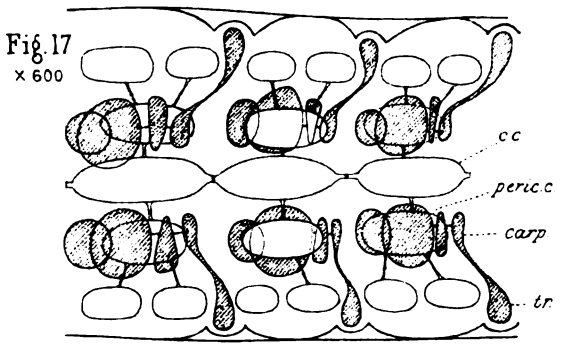


Fig. 17
× 600

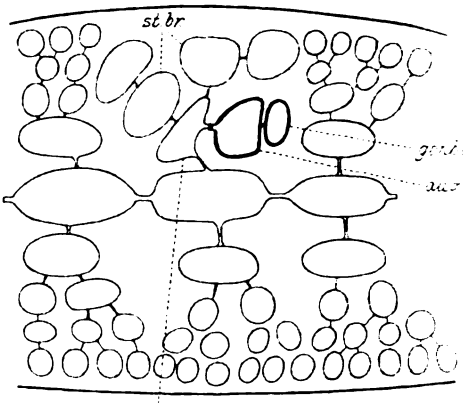


Fig. 18
× 400

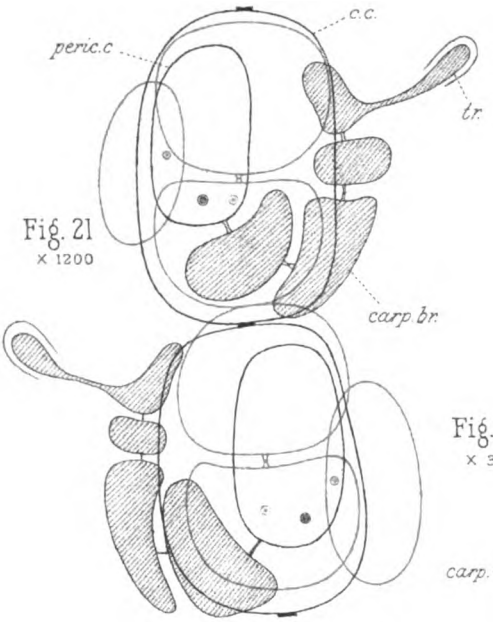


Fig. 21
× 1200

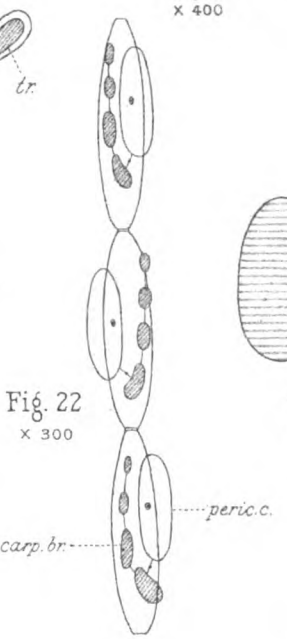


Fig. 22
× 300

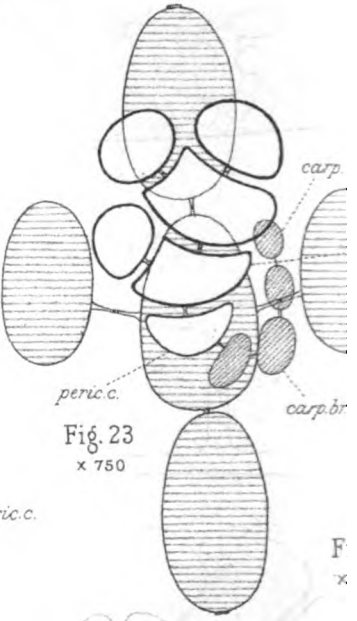


Fig. 23
× 750

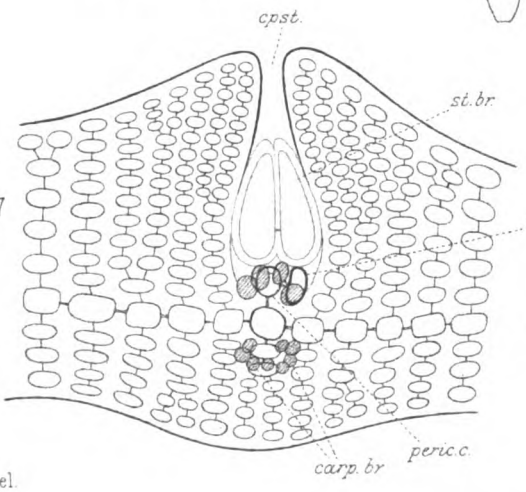


Fig. 27
× 180

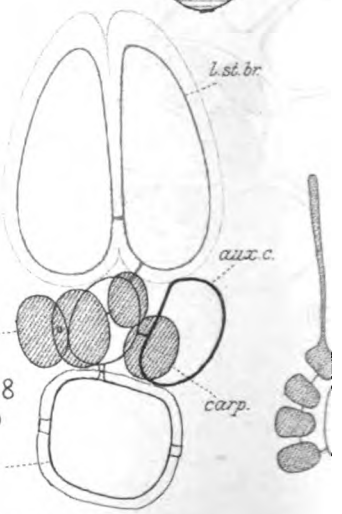
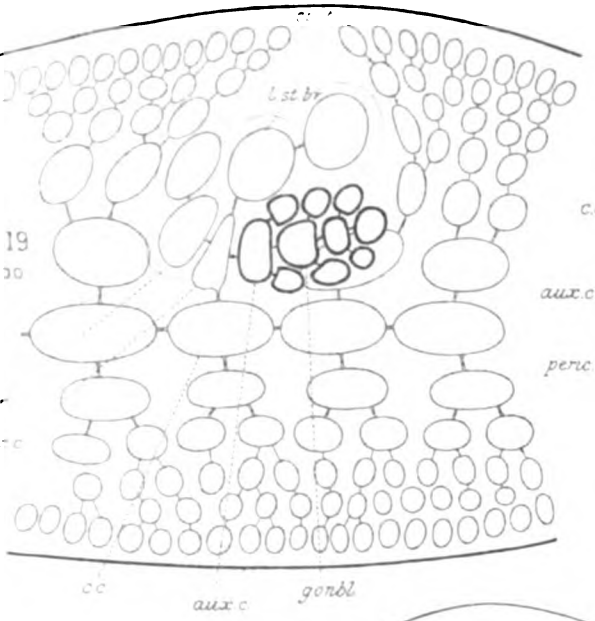


Fig. 28
× 500

W.P. del.

PHILLIPS.— CYSTOCARP IN RHODYMENIALES, II.



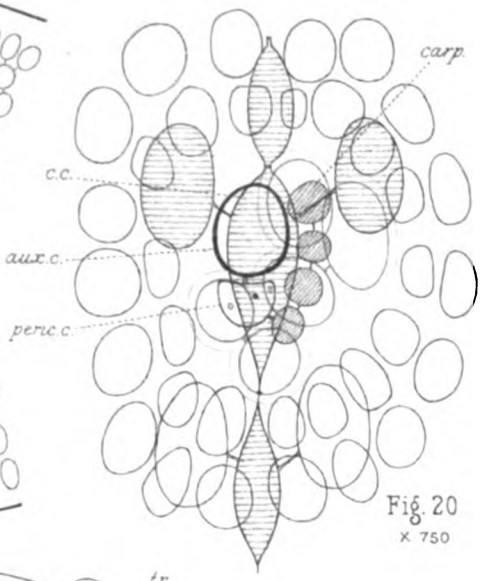


Fig. 20
× 750

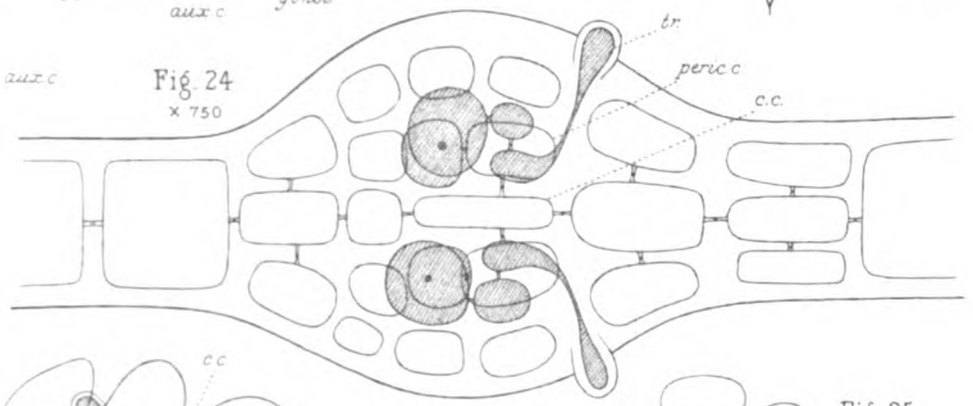


Fig. 24
× 750

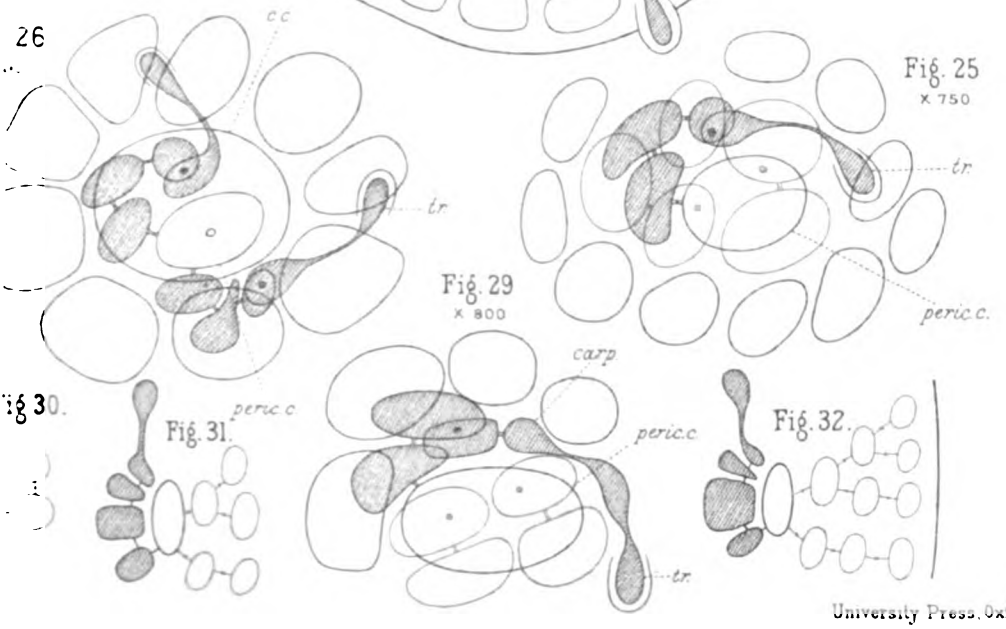


Fig. 25
× 750

Fig. 29
× 800

Fig. 30.

Fig. 31.

Fig. 32.

The Vascular Structure of the Sporophylls of the Cycadaceae[1].

BY

W. C. WORSDELL, F.L.S.

With Plates XVII and XVIII.

ONE of the most marked characters of the plants comprising the order Cycadaceae is the sympodial nature of the development of the stem, whereby the main axis usually terminates in a peduncle bearing a cone with sporophylls, while the vegetative axis is continued as a lateral branch. An exception, however, is found in the female plant of *Cycas*, where sympodial branching does not obtain, the vegetative axis growing continuously through; here the sporophylls, instead of being borne on differentiated portions of the axis known as cones, occur in whorls intercalated amongst the ordinary foliage-leaves of the stem.

The structure of the peduncle in this order has recently been described[2], and has been found to possess certain structures not known in the vegetative axis, such as the primary concentric strands in the cortex and in the central

[1] From the Jodrell Laboratory, Royal Gardens, Kew.

[2] Scott, Ann. Bot., Vol. xi, 1897.

[Annals of Botany, Vol. XII. No. XLVI. June, 1898.]

cylinder, and the centripetally-formed xylem of the bundles composing the latter. These are, I think, ancestral characters which, in the vegetative part of the stem, have been lost owing to special modification of its tissues for functional purposes, and which in the peduncle, as having undergone much less modification in structure, have been retained. This more primitive structure of the peduncle has been emphasized by Solms-Laubach[1] as a result of his observations on the course of the vascular bundles in this organ, and also by Scott[2], who first discovered the presence of centripetal xylem here.

Precisely as in the case of the peduncle, we shall find that the *sporophyll,* the normal foliar appendage of the former, has also retained certain primitive characters which are not found in the foliage-leaf. But in the following pages two factors have to be considered in the structure of the former—the presence of these primitive characters, and the modification of its general structure in connexion with the sporangiferous function.

In order to throw light upon the structure of the sporophyll I shall proceed to compare it with that of the foliage-leaf.

In the base of this latter organ, which is the first-formed portion, the vascular bundles have a purely endarch structure; higher up in the petiole, however, a mesarch structure of the bundle obtains, the centripetal xylem gradually increasing, and the centrifugal xylem gradually decreasing, in quantity from below upwards, until the former is almost entirely predominant. The number of the bundles is almost uniform in every part of the petiole. The difference in structure between the bundles in the leaf-base and those in the remaining portion of the petiole is explicable from the fact that a transition occurs in the bundles of the lower region of the petiole from the structure peculiar to the stem to that which is peculiar to the leaf.

When the structure of the bundle-system in the *sporophyll*

[1] Die Sprossfolge der Stangeria und der übrigen Cycadeen, Bot. Zeit. 1890.
[2] Loc. cit.

is examined, a similar phenomenon, as might be expected, prevails. Here also in the base of the organ, near the point of its attachment to the axis of the cone, the bundles are, or tend to be, endarch in structure, while in proportion as they pass higher up towards the lamina the endarch is gradually replaced by the mesarch character, until, in the lamina of the organ, the latter predominates. Thus the same general structure obtains here as in the foliage-leaf.

Now the sporophyll has a function to perform which is entirely different from that of the foliage-leaf. Whereas the function of the latter is to elaborate food-material for the supply of the various parts of the plant, to aid in the conduction of water through the stem, and to discharge the surplus quantity of this water from its expanded surfaces, the function of the sporophyll is to bear the sporangia, and to act as channels for the conduction of nutriment of all kinds to these latter; it also, in some cases, takes a small part in assimilation.

Though both foliage-leaf and sporophyll are morphologically of foliar nature, and possess essentially the same structure, their physiological relations are seen to be widely divergent. It is the special sporangiferous function of the sporophyll which has induced those modifications in the structure and arrangement of the vascular bundles of this organ which are considered worthy to form the subject of a separate paper.

There exists a considerable range of variation as regards the external development of the sporophyll in the different genera, and sometimes also within the same species between the two sexes. They may be either large and leaf-like or very much reduced and modified in shape, so as no longer to bear much resemblance to ordinary foliar organs.

In the *male sporophyll*, as is known, the sporangia are thickly arranged in sori on the lower surface of a large flattened area situated between the extremely short stalk and the terminal, swollen, sterile portion; the function of the latter is to afford protection to the sporangia within; the apex

of this part is often prolonged into a short acuminate blade, recalling its foliar nature, or it may be produced on each side into two reflexed horns.

The numerous bundles which are found in the stalk of this organ usually have their origin in a single bundle which is given off as a lateral branch from one of the members of the central cylinder of the axis of the cone; this bundle, while traversing the cortex, divides into two, and these in the base of the sporophyll split up at first into three and very soon after into a larger number. Occasionally the two bundles may arise separately and independently from the cylinder of the axis. The bundles of the stalk are usually of small size, their xylem being mainly of *centrifugal* origin, sharply defined and deeply staining with safranin, and often, but not always, accompanied by a (usually) much smaller quantity of *centripetal* xylem, which, however, is not so sharply defined nor so brightly stained, the tracheides of the centrifugal portion being evidently better lignified and chiefly functional in conduction. In proportion as the bundles pass up into the flattened portion of the sporophyll bearing the sporangia, the centripetal increases and the centrifugal xylem decreases in significance and quantity, until, in the sterile portion above, the former predominates. In this latter portion the structure of the bundles is essentially the same as that in the foliage-leaf. The centripetal xylem is as sharply defined as, and often even of a brighter colour, than the centrifugal, and is usually present in greater quantity. The bundles are of very varying sizes; they have no definite orientation, lying in all directions. They arise from successive branching of those in the stalk. They have no longer any important conducting function to perform, the xylem being probably chiefly serviceable as a strengthening framework, so that the phloem is usually reduced in quantity and definiteness. The change of function of the bundles in this region and their relative unimportance will probably have much to do with the inconstancy of structure displayed, for the two kinds of xylem are very unequally developed in different bundles, the one often

entirely supplanting the other, and vice versa. The very frequent presence here of the bundles with an endarch structure probably indicates the far-reaching influence of the special physiological function of the sporophyll, which causes, even in the region where it can no longer have any meaning, the suppression of the centripetal in favour of the centrifugal xylem. In the extreme portions the bundles are often reduced to a few irregular large tracheides. Transfusion-tissue is of very frequent occurrence in connexion with the bundles, and is sometimes of considerable development.

In the *female sporophyll* there are usually about four, sometimes a larger number, or only two bundles to be seen in the stalk. Two bundles arise independently, as a rule, from two distinct members of the central cylinder of the axis; these, where a larger number supplies the sporophyll, divide up in the cortex before entering the latter.

The structure and behaviour of the bundles in the female sporophyll are essentially the same as has been described for those in the male organ; but the difference in sex of the sporangia, necessarily involving a difference in their function, a great reduction in their number, a considerable increase in their individual size, and an altered position on the sporophyll, goes hand in hand with a certain difference in the development of the bundles. The bundles which are told off to supply the sporangia are, in most cases, two in number, one at either side of the much elongated stalk; each of these branches in the base of the expanded portion, sending off one or two bundles for the sporangium, which in nearly all genera is situated at the margin of the sporophyll in the sinus formed by the reflexed lateral portion of this part of the sporophyll, or on the tip of the reflexed portion, so that its apex is directed towards the axis of the cone. There is considerable variation in the number of the bundles which enter the sporangium and in the amount of branching which takes place after the one or two strands have been given off from the bundle proceeding from the stalk. The two lateral bundles in the stalk which supply the sporangia are always

much larger than, often extremely large as compared with, the other intermediate bundles which run right through into the sterile portion without taking any part whatever in the conduction of substances to the sporangia. These latter bundles are always quite small, their xylem and phloem being but poorly developed. The lateral bundles, on the other hand, have a great development of centrifugal xylem and phloem, as would, from their function, be expected. For each one has to supply a large megasporangium, and this over a protracted period, during which the processes of sporangial development, fertilization, and embryonic growth must supervene, demanding a constant and adequate stream of nutritive substances through the tissues of these bundles. Hence is the difference in structure and development between the bundles of one and the same female sporophyll, and between these and the bundles of the male sporophyll, easily explained. These larger bundles very often have an element or two of centripetal xylem, while this tissue is usually absent in the smaller bundles, the large development of the bundle seeming to go hand in hand with the presence of centripetal xylem, as will be seen more clearly when the individual genera are treated of.

The structure of the bundles in the thickened, expanded portion of the female sporophyll agrees essentially with that of the same region in the male organ. An exception to the general structure prevailing here is found in the bundles, or a certain number of them, which enter the megasporangium, and occur immediately below the base of the latter; these bundles assume, in many cases, a perfectly *concentric* structure, which may or may not enclose a central pith, and may be large or quite minute in size; others are not completely but only partially concentric, while others again are quite collateral in structure. All these kinds of structure may occur in the same group of bundles entering the sporangia. These concentric strands I regard not as purely adaptive structures connected with the radial symmetry of the sporangium they supply, for though this idea might explain the structure of

the single large concentric strand entering the central chalazal portion of the sporangium, and which breaks up into a group of bundles radiating from the common centre to the different parts of the organ, it will hardly entirely account for the extremely small concentric bundles occurring in no very definite position in the group, and which must necessarily take a subsidiary part in supplying the sporangium. I am inclined, on the other hand, to regard these *concentric* structures and the constancy with which they occur in the region concerned, as relics of the original primitive structure appearing in what must be considered as the most primitive tissue of the sporophyll.

It is the *function* of the individual bundles of the sporophyll, both on the male and female side, which readily explains their various degrees of development and varying types of structure. The bundles of the male sporophyll, at the level of the insertion of the sporangia, are small and insignificant in appearance because each one is told off to supply one of the numerous small sori scattered over the surface of the sporophyll, the microsporangia composing each having but a very temporary existence, insomuch that the function of the bundle supplying them ceases with the dispersion of the spores. Even in the lowest region of the stalk, before much branching of the bundles has occurred, and where they are therefore somewhat larger and fewer in number, the latter have quite a small development of centrifugal xylem as compared with that of the bundles supplying the megasporangia, this being clearly correlated with the respective functions of these bundles. But why in some genera the bundles in the basal region of the stalk of either male or female sporophyll should possess centripetal xylem, while in the case of other genera this tissue should be quite absent, and why this variation should exist both between the bundles of sporophylls of opposite sexes and between the bundles of one and the same sporophyll, is no more obvious than the reason why the same variation in structure should prevail in the bundles of the peduncle, not only of different genera, but also of different

species, as has recently been clearly observed [1]. The greater or less development of the centrifugal portion of the bundle does not appear to be the sole or even the principal conditioning factor in determining the amount of centripetal xylem present, for where the latter is fairly well represented, the centrifugal portion may be better developed than in those cases where it is absent. There is evidently in these organs, as in the peduncle, the axis of the cone, and the foliage-leaf, a tendency, in spite of other prevailing influences, for the primitive mesarch characters to appear in the bundles, this tendency being stronger in some genera than in others, notably in those in which the sporophylls are of large size, this latter being doubtless indicative of their more primitive character as compared with the smaller and more highly modified sporophylls of other genera.

In the cones of both sexes of all genera there are always, at the base of the cone, a number of sterile sporophylls, which may either have the form of the fertile organs and be crowded together like these, or may be elongated structures more like bracts in shape and position; frequently a gradual transition occurs between these latter and the normal fertile sporophylls. It is interesting to note that the vascular structure of these barren sporophylls, while partaking in a general way of that of the fertile organs, exhibits a more primitive character, owing chiefly to the fact of the absence of the sporangiferous function in these organs, whereby these primitive structures, which consist of the more frequent presence of concentric structures and the occasional abortion of parts of the vascular system, have not been so much interfered with and altered as in the case of the fertile sporophylls. The small bundles in the cortex of the upper portion of the peduncle of both sexes, which are usually observed in pairs, and which supply the sterile sporophylls, possess very often a small quantity of centripetal xylem [2], which is frequently developed as transfusion-tissue both in a ventral and lateral position.

[1] Scott, loc. cit.

[2] Cf. Scott, loc. cit., p. 406.

I will now proceed to describe the structure of the vascular bundle-system of the sporophylls of the various genera of the order.

CYCAS REVOLUTA, Thunb.

Male Sporophyll. The few sporophylls available for investigation were supplied from a male cone preserved in spirit in Museum No. I in the Royal Gardens. On this account the course of the bundles from the central cylinder of the axis of the cone to their entry into the sporophyll was not observed; but this has been adequately followed in *C. siamensis* Miq. and *C. circinalis* L. by Thibout [1], who finds that a single bundle enters the cylinder from the sporophyll. The sporophyll does not assume such a perpendicular position with regard to the axis of the cone as in most genera, but is more or less curved. It is also larger, not being quite so reduced in size as in other cases. There are a number of bundles in a row whose size is in correspondence with that of the sporophyll. They possess sharply defined centrifugal xylem in fair quantity. Most of them have an almost equal quantity of centripetal xylem; but the latter, in one or two cases, far exceeds the centrifugal, consisting of a great number of elements extending some distance from the protoxylem. The tracheides of the centripetal are often far larger than those of the centrifugal xylem. But in every case the centripetal is less brightly and sharply stained than the centrifugal portion, this fact indicating that the latter is more strongly lignified and thus chiefly functional in conduction.

The chief feature of the structure of these male sporophylls of *Cycas* is the appearance, in the bundles of the lower portion of the stalk, of centripetal xylem. In no other genus is this tissue so well-developed in this region of the sporophyll, and it affords an instance of the way in which the centripetal tracheides may form the chief part of the xylem quite low down in the stalk. In the sterile portion the centrifugal

[1] Recherches sur l'Appareil Mâle des Gymnospermes, 1896.

xylem of the bundle is greatly reduced in quantity, its elements less sharply defined, and less brightly stained. The centripetal xylem usually exceeds it in quantity.

Female Sporophyll. This organ is situated on the main axis of the vegetative stem, and not on a strobilus as in all the other genera. It occurs in whorls alternating with the foliage-leaves. It is larger in size than the sporophyll of any other genus, and more nearly resembles a foliage-leaf in external conformation; herein lies probably a more primitive and less modified character; the sporophylls of other genera, from their crowded arrangement on a cone, having undergone a much greater modification in form. These sporophylls differ also from all others in possessing a very long stalk, and in bearing a larger number of megasporangia than two, these being situated, not on the terminal expanded portion of the sporophyll, as in other genera, but at regular intervals on short projections from the stalk. The size of the sporangia is in correspondence with that of the sporophyll, being the largest of the whole order.

The structure of the vascular bundles of the sporophylls is remarkable and interesting; it is precisely that which is found in the bundles of the peduncle of *Stangeria*. The strands, as seen in transverse section, are arranged laterally in groups which form a single row (Fig. 1). Some bundles or groups of bundles are entirely or partially surrounded by a thick zone of cells, probably pericyclic, filled with dense protoplasmic contents and conspicuous nuclei. This, again, is surrounded by a belt of stone-cells, possibly representing an endodermis, which have conspicuous reticulate thickenings on all their walls. But these two tissues are of inconstant appearance.

The *centrifugal* xylem and the phloem are of very great development, which is quite equal to that of the bundles in the peduncle of other genera, and is in evident correlation with the size and number of the sporangia which they have to supply. The *centripetal* xylem, either in contact with the protoxylem, or as scattered tracheides or groups of

tracheides lying some distance away, is always present; in position and development it is the exact counterpart of that in the peduncle of *Stangeria*. It sometimes occurs opposite a gap between the bundles.

Transfusion-tissue, of quite small tracheides with bordered pits or close reticulations, occurs in proximity to the centripetal xylem both in a ventral and lateral position, and its derivation from this tissue is obvious. It is present in connexion with the cells containing dense protoplasmic contents surrounding the bundle; where these cells are absent the transfusion-tissue is also no longer to be found. Some of the bundles are curved into a semi-concentric shape. On the dorsal side of nearly all the bundles are small strands with inverted orientation, their phloem being directly in contact with that of the main bundles. Each has an active cambium with (usually) a fair amount of xylem and phloem developed. Some have protoxylem; other smaller ones have none, so that they are not all secondary. In this respect they resemble the similar strands observed in the peduncle of *Ceratozamia*. In one case, on the dorsal side of the protoxylem, i.e. on the side towards the periphery of the sporophyll, two tracheides occur which have evidently been formed by a cambium centrifugally, for regular radial rows of cells are seen on the dorsal side of these two tracheides. Also, on the ventral side of some of the tracheides of the centripetal xylem of the main strand, evidence of cambial divisions occurs such as I have also seen in the peduncle of *Stangeria*; these were always observed in sister-cells of such tracheides lying on the inner (ventral) side of the latter towards the pith. Such division-walls were noticed nowhere else in the surrounding parenchyma-cells.

Two strands are observed to pass off to a sporangium and enter it without previous branching. One of these is almost completely *concentric* in structure; it has a large amount of secondary centrifugal xylem, and about three groups of protoxylem are observed around the inner margin of the latter. In the greater part of its contour the strand is

evidently of primary origin, but a small segment of the cylinder is probably formed of purely secondary tissue, though this is not perfectly certain; but no crushed phloem-elements are observed at the periphery of this part. In the central parenchymatous tissue, or pith, of the strand occur a few tracheides isolated or in groups, some of which are attached to the protoxylem; they do not extend to the centre, but quite a small central free space is left; they evidently belong to the primary centripetal system (Fig. 4).

This concentric strand, for about three-fourths of its contour, has small inverted strands attached to it, whose tissues appear to be mostly secondary; there is no sign of primary phloem anywhere, no crushed elements of the latter being visible, most of the elements in this region occurring in the same radial rows with cambial cells. But the xylem of these strands, besides the numerous layers of secondary tracheides, has in all cases some primary xylem, some of the elements being small, others large and developed as transfusion-tissue with reticulate thickenings on the transverse wall, which occur both in a lateral and a ventral position, often in great quantity. In only one of these small bundles could I discern any sign of protoxylem, in most this tissue appears to be absent. In one or two cases there seems to be a considerable quantity of primary centrifugal xylem. In the tissue below the place of insertion of other megasporangia the strands are quite collateral, with a few centripetal tracheides, of which, in one instance, a radial row of three was attached to the protoxylem; a sheath of stone-cells extends round close to this row of tracheides. In some of the bundles a large amount of transfusion-tissue runs out amongst the cells with conspicuous nuclei and dense protoplasmic contents occupying the region between the strands and the sheath.

In the sterile region of the sporophyll, above the insertion of the sporangia, the bundles are much reduced as regards their centrifugal xylem and their phloem, so that the former approximates more to the dimensions of the centripetal xylem,

while in the pinnae of the organ the bundles undergo a still greater reduction, and resemble there in their structure those of the ordinary foliage-leaf.

CYCAS CIRCINALIS, L.

Female Sporophyll. In this plant the same structure of the strands prevails, but the latter are usually much elongated tangentially (Fig. 2). Some of these tend to become revoluted towards the dorsal side, a phenomenon similar to that occurring in the root of *C. Seemanni*, Al. Br.[1]

A single strand passes off to the sporangia, and in so doing divides into 3–5 strands which enter the latter; some of those have a perfectly concentric structure. Fig. 3 represents a bundle from the lamina in transverse section.

The sporophylls are much more slender and the sporangia smaller than in the last species.

STANGERIA PARADOXA, Th. Moore.

Male Sporophyll. In the stalk are a number of bundles in a row (except at the very base where there are only two). In the well-developed centrifugal xylem the tracheides are clear and well defined, taking the stain brightly. Centripetal xylem is quite absent. In the sterile portion the centrifugal xylem is much reduced, its elements being inconspicuous and not so brightly stained as in those of the stalk; in many bundles one, three, or four elements of centripetal xylem occur, in other bundles there are none seen at all.

Female Sporophyll. Two bundles leave the cylinder of the axis of the female cone, each from a separate strand, and may either enter the sporophyll without dividing further, furnishing to that organ two large bundles of equal development, or they may previously divide so as to form three or four bundles, of which the outer one on each side supplying the sporangium is considerably larger than the one or two

[1] Gregg, Ann. Bot., Vol. i, p. 4, Fig. 1, 1887.

intermediate ones (Figs. 6 and 7). Only in one or two bundles are two or three very small centripetal tracheides seen. The bundle at either side which supplies the sporangium forks just on entering the base of the lamina, one branch passing upwards, the other bending off towards the reflexed lateral lobe, between which and the stalk the sporangium is seated. Before this bundle assumes its downward course into the lobe, however, and opposite the place of insertion of the sporangium, it gives off a branch which forks: of the two bundles thus formed, one enters the sporangium without further change, the other, immediately before entering the latter, divides into two (Fig. 5).

The bundles of the *lamina* are characterized by a great reduction in the centrifugal and a corresponding increase in the centripetal xylem, so that the two parts are about equal in development (Fig. 8). The lamina of a female sporophyll at a very young stage was examined; in this is a number of very small bundles, most of which have an element or two of centripetal and one or two of centrifugal xylem. A few of them have no centripetal xylem besides the protoxylem. It is difficult to make out which is primary and which secondary centrifugal xylem in some bundles; the smaller elements in connexion with the protoxylem are probably primary. The centrifugal and centripetal xylem appear to be developed simultaneously. Many of the elements of the latter are very small and closely united to the protoxylem, two or three together, and would most likely have the same spiral thickenings as the latter; others usually single, lie some distance away.

Scale Leaves and Barren Sporophylls. At the base of the peduncle of the male cone are large, narrow, elongated scales, consisting of a fleshy central portion, with a wing on either side. They contain at the base three bundles which are collateral in shape, and possess secondary centrifugal xylem; a tracheide belonging to the centripetal xylem is here and there seen. In the upper part of the scale the bundles increase to four or six, becoming very much reduced in size,

and exceedingly small. They are here seen to possess one or two centrifugal and one or two centripetal tracheides; there is a very small quantity of phloem; no definite cambium can be distinguished, so that it is possible that the centrifugal tracheides are primary. The bundles are arranged in an arc. Sclerotic cells are scattered about in the ground-tissue.

The uppermost scales are more leaf-like and much broader than the lower scales, and represent sterile sporophylls, resembling in shape the fertile organs. One of these, whose structure was examined, has an extremely long foliar base, running ridge-like, a considerable distance down the peduncle. About half way up this foliar base a minute concentric bundle becomes differentiated from the ground-tissue, arising quite independently of any other strand (Fig. 10). Higher up it gradually increases in size, and in the upper free part of the organ is observed to give rise to an imperfect branching system forming the nervation of one side of the sporophyll. About three or four branches occur, all very imperfect, interrupted for considerable spaces in different parts of their course, and dying out before reaching the margin of the sporophyll; they appear to have a very reduced structure, and their tracheides are much twisted and contorted. From their evident progress towards extinction and the concentric structure of the bundle from which they spring, they may be said to represent part of the original primitive bundle-system of the sporophyll which has long since ceased its connexion with that of the peduncle. A portion of the system, however, has still retained this connexion, and after a large part of the sporophyll became fused with the peduncular tissues, the bundle constituting the base of this system assumed a more direct course into the axis, passing in in the upper part of the foliar base, instead of, as in the case of the small concentric bundle, running down in the latter towards its lower extremity. This bundle, which enters the barren sporophyll from the peduncle, possesses, whilst still in the latter, two distinct and well-marked groups of centripetal xylem. The subsequent course and branching of this bundle

and the nervation to which it gives rise, though imperfect, are not nearly so rudimentary and reduced in character as the one above described, for the bundles composing it are perfectly functional and nearly everywhere continuous; the tip of the organ is occupied by 3–5 bundles formed by the division of one of the two main branches of the bundle coming from the axis; these bundles have the structure characteristic of those in the sterile portion of the sporophyll, while the large bundle in the lower part has an endarch structure. The presence of these bundles in the cortex supplying the barren sporophylls was observed in two or three female and also in several male peduncles; they were most abundant in the former, where they often occur in longer or shorter and very straight tangential rows in the outer part of the cortex, but may also occur quite isolated. Most of these bundles possess, besides the usual endarch portion, two, often very large, groups of centripetal xylem, lying each slightly to one side of the median ventral line, and with tracheides of which those farthest towards the ventral side are three or four times the size of those composing the centrifugal xylem. Protoxylem is seen to be attached to each group (Fig. 9). But there is considerable variation in the development of these centripetal xylem groups, for they may be very much more reduced than those shown in Fig. 9, or even almost entirely absent; they may be also in much closer connexion with the centrifugal xylem, or even, as in the case of one such group belonging to a bundle in a male peduncle, *perfectly continuous therewith.*

The reason why I attach special importance to these bundles arises from the fact that I regard each such bundle, with its three distinct groups of xylem, as the vestige of a primitive *concentric* leaf-trace bundle which was probably characteristic of the ancestors of these plants. Now in the cortex of the stem of certain species of *Medullosa* just such concentric leaf-traces are known to occur, which during their course outward become split up into three portions, the concentric structure becoming thereby lost. My Figure 9

may be aptly compared with Fig. 8 (in the text) of Weber and Sterzel's work[1], and with Fig. 9, Plate V of Solms' paper. It will be seen that in the bundles of the fossil plants secondary xylem is present in all three parts in considerable quantity, while in those of modern plants this tissue occurs in small quantity in the complete dorsal portion only. In a male peduncle of *Stangeria* one of the bundles with its two groups of centripetal xylem was traced into the elongated barren sporophyll, as above described, when the centripetal xylem-groups were found to gradually die out as the bundle passes into the sporophyll, so that a purely endarch structure of the strand remained. I regard the two ventral groups of xylem each with its inner protoxylem-strand, as the remnants of two distinct bundles, whose phloem has disappeared and which are now more or less separated from each other and from the dorsally-placed complete bundle with which, in the ancestors of the plant, they formed a compact whole as a concentric leaf-trace bundle.

In the same female peduncles groups of three bundles some little distance apart, with their xylems converging, are often seen close to the central cylinder. These appear to me to represent, in a rather different way, the same phenomenon of a vestige of an ancestral concentric strand; in this case, however, the three parts have not become in any way reduced, but they have, however, undergone much more complete separation from each other than in the previous case.

BOWENIA SPECTABILIS, Hook.

Male Sporophyll. A single bundle leaves the vascular cylinder of the axis of the male cone and very soon, almost immediately, divides into two. These run through the cortex and enter a sporophyll, where they forthwith begin to divide up into a number of bundles.

In the stalk of the sporophyll the bundles are nearly all

[1] Weber u. Sterzel, Beiträge zur Kenntniss der Medulloseae, Chemnitz, 1896; pp. 17 and 18, Figs. 7 and 8 of text, and Plate III. Solms-Laubach, Ueber Medullosa Leuckharti, Bot. Zeit., 1897.

extremely minute, with centrifugal xylem only, the tracheides of which are well defined and thick-walled. The bundles are very irregular in position and orientated in different directions. In the sterile portion the centrifugal xylem is much reduced and quite insignificant. The centripetal xylem is fairly well developed and its tracheides much scattered and wandering. In some of the bundles the phloem is scarcely developed, and the parts of the xylem cannot be properly orientated.

Female Sporophyll. Two bundles leave the central cylinder of the axis of the female cone; in the outer part of the cortex each of these divides into two, so that four bundles are produced. In those cases where other bundles, besides these four, occur, in the stalk of the sporophyll, these are cut off by the two original bundles just before entering the latter, and passing off towards the ventral side of the sporophyll, they, in the majority of cases, twist on their axes, assuming thereby a more or less abnormal orientation (Fig. 11). The number, orientation, and structure of these ventral bundles in the stalk varies greatly; the following are the principal cases met with:—

1. One large bundle with inverted orientation.

2. Three bundles, two with inverted orientation, and the median one concentric (Figs. 11 and 12).

3. Two bundles, closely contiguous, lying sideways with their xylems facing each other.

4. Two bundles, one of which lies sideways, the other obliquely.

5. One bundle, lying close on the ventral side of one of the normal bundles, and with normal orientation, but placed rather obliquely.

6. One bundle, lying sideways.

7. One bundle, with normal orientation.

All the ventral bundles pass up into the lamina, without taking any part in supplying the sporangia; those which in the stalk were inversely orientated, in the lamina twist on their axes once more to assume approximately the normal orientation. Here the same change as regards the relative

development of the centrifugal and centripetal xylem occurs as in previous cases.

Of the four normally orientated bundles in the stalk, the two lateral ones which supply the sporangia are considerably the largest. One or two small elements of centripetal xylem may occur in any of the four bundles, but are nearly always present in the large lateral ones. From each of these latter, as they enter the lamina, two bundles branch off to the sporangium; and each of these, immediately before entering the latter, may divide up into two or three. In transverse section of the part immediately below the insertion of the sporangium, one of the two bundles is seen to be divided into three.

Scale Leaves and Barren Sporophylls. At the base of the male cone are a number of scale leaves similar to those in *Stangeria*. They contain a row of many bundles with endarch structure and very small in size; a few bundles also occur on the ventral side of this row and appear to be normally orientated. Immediately below the compactly-arranged sporophylls of the male, are two or three elongated, barren sporophylls (Fig. 13). In the case of one cone, each barren sporophyll contains, in its lower portion or stalk, a row of four or five bundles, of which the two end ones of the row are concentric in structure (Fig. 14), one of these being very small, the other of normal size. The intermediate bundles are rather larger in size than those of the fertile organ, this being due, possibly, to the smaller number of the former; their structure is similar, the centrifugal xylem being sharply defined and the phloem well developed; in the upper part, or lamina, the smallest concentric bundle dies out, while the larger one assumes a collateral structure; while, as regards the other bundles, a similar change in their structure takes place as in the fertile sporophyll.

DIOON EDULE, Lindl.

Male Sporophyll. In the narrow stalk is a single row of bundles. All are of quite small size and of nearly equal

development as regards the centripetal xylem, which is quite small in amount. Nearly all the bundles have three or four (one bundle had seven or eight) tracheides of centripetal xylem; these are, however, always faint, rounded, and weakly stained compared with those of the centrifugal xylem. In the sterile portion the usual structure of this part prevails; some of the centripetal xylem is developed as transfusion-tissue which is both lateral and ventral in position.

Female Sporophyll. Two bundles leave the cylinder of the axis of the female cone, and on their way through the cortex divide up into a considerable number of bundles, so that in the stalk of the sporophyll a row of about nine bundles occurs. Though the sporophyll is one of the largest in the order (equal in size to that of *Encephalartos*), the mega-sporangia are not nearly so large as in that genus; consequently, the bundles have also a considerably less development, and are all regularly orientated. In those sporophylls which bear two well-developed sporangia, the one or two bundles at either end of the row are larger and have better-developed phloem and centrifugal xylem than the intermediate ones (Fig. 15). In those with one abortive sporangium, the most external bundle of the row on the same side of the stalk is scarcely better developed than the rest. In those with both sporangia abortive, the same applies to the external bundle on both sides. The bundles of the lamina exhibit the ordinary structure (Fig. 16). The external bundle of the row on one side of the stalk and a branch from the bundle next to it bend off in the lamina and pass down to supply the sporangium; each of these divides into three, so that six bundles enter the latter (Fig. 17). Of these two or three of the smaller ones are perfectly *concentric* in structure. Some of the bundles of the inner portion of the integument of the sporangium have also a *concentric* structure, the protoxylem occupying the centre of the bundle and enclosed by primary tracheides; the phloem, however, on the ventral side is rudimentary in character; the occurrence of these bundles in the integument seems to show that the

concentric structure of those occurring in the sporophyll below the insertion of the sporangium and in the base of the latter is not entirely due to the radial symmetry of the sporangium which they supply.

ENCEPHALARTOS VILLOSUS, Lehm.

Male Sporophyll. In the winged stalk is a row of bundles, of which the one on either side situated between the wing and the central region is the largest, and has a large amount of centrifugal xylem; a certain small amount of centripetal xylem occurs at the sides of the bundle and is continuous with the centrifugal portion. The smaller bundles in the rest of the stalk have also very conspicuous centrifugal xylem of fairly large elements.

Female Sporophyll. The axis of the cone to which these sporophylls were attached was not available for investigation, so that the course of the bundles therein could not be traced.

The sporophylls are the largest of any in the order except those of *Cycas*. Their vascular system is, consequently, very well developed, and much resembles that of *Cycas*. As in that genus, the bundles have a large amount of centrifugal xylem, and many possess besides a considerable quantity of centripetal xylem, though others have less of the latter or even none at all. As in *Cycas* also, there are inverted strands on the dorsal side of many of the bundles, some of which have a small amount of centripetal xylem, while others have none; most, if not all, appear to have protoxylem; two strands placed back to back may be of equal size. But the bundles have a very irregular arrangement, being orientated and grouped in all kinds of ways, resembling somewhat the arrangement of the bundles in the basal region of the peduncle in *Stangeria*. At each side of the stalk there is an aggregation of bundles in close proximity and with variously orientated parts; these are the strands which supply the sporangium; the other bundles are smaller. In shape the bundles of the sporophyll are rounded and often very much curved, so as to

form an almost *concentric* structure (Fig. 18); indeed, in another species, *E. horridus*, Lehm., a primitive concentric structure for these bundles appears to be indicated by the peculiarity presented by some of them, as, for instance, one bundle which is curved into a horse-shoe shape so that the xylem from opposite sides almost meets at the open part of the strand so as to enclose a pith with protoxylem round its periphery; in another larger, tangentially-elongated strand which is but slightly curved, there occur on the ventral side of two groups of centripetal xylem, and intimately connected therewith by radial rows of cells, two distinct groups of crushed phloem-cells, with a slight indication of these latter having at one time been united by similar tissue; thus the combination of this phloem with the groups of centripetal xylem affords the phenomenon of two small strands with inverted orientation on the ventral side of the main strand; other groups of centripetal xylem occur belonging to the same strand, but isolated and without any sign of phloem near them (Fig. 19).

In the *lamina* the strands have about the same general structure; but here the centrifugal xylem is extremely reduced as compared with that in the stalk, its tracheides also being usually much smaller; it is about equal in development to the centripetal which occurs in considerable quantity, and in some bundles extends as transfusion-tissue, with scalariform thickenings on the transverse walls of the tracheides, both laterally, and even quite on to the dorsal side of the phloem. In *E. horridus*, Lehm., protoxylem was only seen on the side of the centripetal xylem.

The megasporangium receives 4–5 bundles, some of which have a very distinct *concentric* structure (Fig. 20).

Macrozamia Fraseri, Miq.

Male Sporophyll. There are about a dozen bundles in the stalk lying in a regular row and somewhat unequal in size. They all have sharply-defined and brightly-stained centri-

fugal and an entire absence of centripetal xylem. They arise from the branching of three bundles (which really represent two, of which one has undergone premature division) which enter the sporophyll from the axis of the cone, these three originating in the cortex from the single bundle which leaves the central cylinder. Owing to this premature division of one of the bundles entering the sporophyll, there are at first a larger number of bundles on one side of the organ than on the other.

In a rather young male sporophyll of *M. spiralis*, Miq., there are about nine bundles in the stalk.

Female Sporophyll. This is one of the large types of sporophyll. Owing to the fact that the material at my disposal was dead and withered, the arrangement of the bundles in the stalk could not be ascertained, as the sections broke up into small fragments. The strands appear to be of the same type, in form and structure, as those in the female sporophyll of *Encephalartos*, as they possess a large amount of centrifugal and also a small but well-marked quantity of centripetal xylem. In the lamina some of the bundles are a good size, the centrifugal and centripetal parts of the xylem being of about equal development, though the former seems to stain rather more sharply than the latter.

ZAMIA LATIFOLIA, Lodd.

Male Sporophyll. In the lower part of the stalk are three bundles with the typical centrifugal and no centripetal xylem. A little higher these divide up into a rather larger number, of which one from each side is seen to pass off to the dorsal side of the sporophyll. One of the bundles in this region show a good example of primary centrifugal xylem; it has a single, minute, flattened tracheide of secondary centrifugal xylem separated from the other portion of this tissue by a layer or two of roundish parenchyma-cells; next, towards the ventral side succeeds a row of four or five tracheides, very well-defined, and with brightly-coloured walls, and then

a second row of as many, but rather smaller and less clearly-defined tracheides, these two constituting the primary centrifugal xylem; these tracheides do not lie in the same radial rows as the cambium-cells further out, but are separated therefrom by the parenchymatous cells afore-mentioned, some of which overlap in position two of the cambium-cells. In the sterile portion the mesarch structure of the bundles is very pronounced.

Female Sporophyll. In the stalk, besides the four bundles which exhibit the usual structure and no centripetal xylem, there is a very small one on the ventral side with inverted orientation and almost concentric structure, which has evidently sprung from one of the four bundles opposite to which it lies.

ZAMIA PUMILA, Linn.

Male Sporophyll. In the stalk is a row of about six bundles, quite small in size, one or two having only a single tracheide of centrifugal xylem. Of these small bundles, two occur on the dorsal side of the row, one of them lying sideways and being semi-concentric in shape. That there is a tendency for a concentric structure to appear in the bundles is shown also by one in which a rudimentary phloem occurs on the ventral side of the protoxylem. In one of the bundles two elements of centripetal xylem occur.

In the sterile portion the bundles have the usual mesarch structure.

ZAMIA SKINNERI, Warsz.

Male Sporophyll. In the stalk are three bundles with well-developed secondary tissue, of which the tracheides, especially in the median bundle, are of considerable size and very brightly coloured. To each bundle a single tracheide of centripetal xylem is seen to be attached. In the region just above where the bundles pass off to the sporangia, occur two large bundles with well-developed, typical mesarch

structure, both the centrifugal and centripetal xylem having each its distinct group of protoxylem. Higher up in the sterile portion these bundles split up into a larger number, some of which are partially concentric in structure like those in the female sporophyll of *Encephalartos*.

ZAMIA MURICATA, Willd.

Male Sporophyll. There are three very small bundles in the stalk, with the usual structure, the two outer ones of which have a sidelong position. But at a certain level there occur in close proximity to two of these the rudiments of a bundle lying obliquely towards the ventral side of the normal bundle; in one case the rudimentary bundle appears to have a mesarch structure, a minute protoxylem-group intervening between the reduced phloem and two rather scattered tracheides. In the sterile portion many of the bundles exhibit a structure intermediate between mesarch and endarch, tracheides occurring at the sides of the protoxylem of which it is not easy to say whether they belong to the centrifugal or centripetal portion of the xylem; in the same region are other bundles with a definite mesarch structure, some of which have no centrifugal xylem at all.

ZAMIA LINDENI, Regel.

Male Sporophyll. In the stalk is a short row of four bundles which are normal in every respect (Fig. 21). In the sterile portion are a great number of bundles orientated in every direction and with the usual structure.

Barren Sporophyll. The smaller compactly-arranged barren sporophylls at the base of the male cone, which are similar in conformation to the fertile organs, present a very interesting structure. The bundles in the stalk, sometimes one, sometimes several in a row, are all more or less to be interpreted as having a partially *concentric* structure; in some

cases the appearance is as if two distinct bundles were closely united, but not completely fused, by their ventral faces; in others the bundle has a curved, or horse-shoe shape. The bundles are all irregularly orientated, mostly assuming a sidelong position (Fig. 22). But these structures are of importance from the fact that they exhibit more primitive characters than the bundles of the fertile sporophyll, the latter differing entirely therefrom, both in structure and arrangement, being perfectly collateral and normally orientated. It is owing to the absence of the sporangiferous function in these barren sporophylls that this primitive structure has been retained.

ZAMIA LODDIGESII, Miq.

Female Sporophyll. Two bundles leave the cylinder of the axis of the female cone, each from a distinct strand thereof. On their way through the cortex each divides into two, thus forming four bundles. The small bundles which occur in pairs in the cortex of the peduncle and which supply the barren sporophylls at the base of the cone have, many of them, centripetal xylem, of the normal kind and in the form of very distinct transfusion-tissue, both in a ventral and a lateral position (Fig. 24).

Of the four bundles in the stalk of the sporophyll, the smaller, newly-formed ones move somewhat forward towards what becomes the ventral side of the organ. The two lateral bundles are somewhat larger, though not so strikingly so as in other genera. All these bundles have several elements of centripetal xylem which are often larger than the tracheides of the centrifugal portion; in this respect this plant affords quite an exception to the general rule.

One bundle turns off to the megasporangium on each side, dividing into two main branches; one of these, the proximal one, before entering the sporangium, divides in different directions into three branches; the distal bundle, which

passes obliquely away to the far side of the sporangium, remains undivided. As seen in transverse section, a bundle about to enter the sporangium has a curved contour, with very well-developed centrifugal and often a large amount also of centripetal xylem; the occurrence of this latter is perhaps correlated with the evident tendency of these bundles to revert to a concentric structure.

In many of the bundles in the most distant portions of the lamina, transfusion-tissue is very markedly developed, resulting from the extension, even on to the dorsal side of the phloem, of the centripetal xylem (Fig. 23). As in nearly all other cases, the tracheides of the centrifugal are always much smaller than those of the centripetal xylem.

ZAMIA FURFURACEA, Ait.

Female Sporophyll. In the stalk are four bundles with the usual structure; no centripetal xylem occurs here; the two lateral bundles are rather better developed than the others and have larger centrifugal tracheides. A little higher up in the sporophyll one of the middle bundles gives off a very small branch which gradually, in passing upwards and towards the ventral side, turns on its axis, assuming directly afterwards a perfectly *concentric* structure, which again, higher up, opens out and becomes collateral; there may be more than one bundle on the ventral side of the normal row; these bundles pass up into the lamina without again assuming a normal orientation. In this latter region of the sporophyll transfusion-tissue is well developed in many of the bundles.

Two bundles enter each megasporangium; one of these, the largest, being the lateral, unbranched bundle of the stalk on the same side which passes up round the axil of insertion of the sporangium; the other, which is very much smaller, as a descending branch from a bundle which has passed a considerable distance up into the lamina; there is thus

a difference in this respect from what occurs in the case of *Z. Loddigesii*. The smaller bundle is collateral in structure and somewhat curved in shape, with two or three elements of centripetal xylem, the other larger one has well-developed centrifugal and no centripetal xylem, and is curved inwards so as to assume an almost *concentric* structure; in the base of the sporangium this bundle begins to separate into two, as a result of which two almost distinct bundles come to lie in close contact, with their xylems facing each other.

ZAMIA FISCHERI, Miq.

Female Sporophyll. The arrangement and structure of the bundles here is very similar to what obtains in the last plant; a small ventral, inverted bundle also being present.

ZAMIA LEIBOLDII, Miq.

Female Sporophyll. The sporophyll examined bore *three* megasporangia (Fig. 25); as a consequence of this the arrangement and structure of the bundles in the stalk are somewhat modified to suit this abnormality. On the side on which the two sporangia occur is a bundle much larger than is usually the case, and close to it another bundle almost or quite as large, but lying somewhat obliquely and with inverted orientation. The bundle at the other side of the sporophyll is very much smaller, corresponding more in size to the bundles supplying the sporangia in previous species. At a slightly higher level the inverted bundle on the side of the two sporangia turns on its axis and becomes united laterally, in the normal position, with the adjacent bundle, so as to form a single exceptionally large bundle, considerably elongated in the tangential direction, and with very well developed centrifugal but no centripetal xylem. The unusual size of this bundle is obviously correlated with the extra sporangium attached to that side of the sporophyll.

At a somewhat higher level still, this large strand divides up into a row of four, of which the two end-bundles are larger, and have their centrifugal xylem developed in such a way as to indicate that they are the bundles which are to supply the sporangia. Hence, probably on account of the lop-sided character of the sporophyll, owing to the extra sporangium being present on one side, the vascular system, to counterbalance this, is concentrated on this side, the normal row of four bundles, instead of being evenly spread over the tissues of the sporophyll, as in all other cases, is limited to the side on which this extra sporangium occurs. The smaller bundle on the other side of the stalk has nothing to do with any of the sporangia, but passes up into the lamina. In the upper part of the stalk about three smaller ventral bundles are seen, one of which is *concentric* in structure, the others irregularly orientated; tracing these downwards, one of them is seen to spring from one of the four bundles of the row, the other two, of which the concentric one opens out and becomes collateral, end blindly each close up against the rim of a mucilage-canal. Higher up, the two end-bundles of the normal row are seen to pass off respectively right and left to the sporangia (the one supplying the two sporangia much earlier than the other). The extra sporangium may be fed by subsequent branching of the bundle passing off to the normal sporangium on that side; but the exact course of the bundles to each sporangium was not followed. The bundle passing off to supply the single sporangium on the other side appears to give off a small branch while doing so; this does not happen in the case of the bundle supplying the two sporangia. The large bundle found entering the sporangium is almost concentric, as in other cases; on entering the sporangium it splits up into a number of parts which radiate out from the common centre.

Fig. 25 *a* and *b* represent respectively ventral and lateral views of the sporophyll.

CERATOZAMIA LATIFOLIA, Miq.

Male Sporophyll. The course of the bundles from the central cylinder of the axis of the male cone to the sporophyll has been thoroughly described by Thibout in *C. mexicana*, Brongn.[1] The occurrence of medullary bundles in the axis has also been mentioned and figured by him[2], and by Scott[3] in that species. In the present species I observed in one case three small bundles lying in the pith immediately within the normal ring which, one after the other, end blindly upwards. In another case a single large bundle in a similar position was traced upwards for a long distance; in one part of its course it split into two bundles which directly afterwards again fused together into one. It twice partially fused with a bundle of the ring during its course, but was not seen to completely fuse therewith. A similar large bundle, which at first was collateral, became higher up *concentric*, and at a still higher level again collateral; it eventually ended blindly in the pith. These characters are confined, in this species, to the male cone; they do not, however, occur in the male cone of *C. Miqueliana*, H. Wendl.

In the lowest part of the stalk of the sporophyll three bundles occur, of which the median one is much smaller than the others; the centrifugal xylem is clearly defined and brightly coloured, and there is no sign of any centripetal xylem. For the rest, the structure is similar to that of the bundles in the male sporophyll of other genera.

CERATOZAMIA MEXICANA, Brongn.

Male Sporophyll. In the lowest part of the stalk are three bundles, the median one as in the last species, being much smaller than the others, and lying out of the row or a short distance towards the dorsal side (Fig. 26). In the sterile

[1] Loc. cit. p. 23. [2] Loc. cit. p. 24. [3] Loc. cit. p. 412.

portion of a young sporophyll in which the parts of the bundle are not as yet fully developed, the xylem consists of protoxylem, on the ventral side of which are two or three elements of centripetal, and occasionally, on the dorsal side, one or two elements of primary centrifugal xylem, and in the lateral region are primary elements intermediate between the two kinds. In one or two bundles one tracheide was seen which appears to be secondary in origin, and which was deeper in colour and larger than the others, and isolated, lying quite apart from the group of primary tracheides.

Female Sporophyll. Two bundles arise independently from a distinct strand of the central cylinder of the axis of the female cone; on their way outward through the cortex they each divide up into a number of bundles, some of which pass up on to the ventral side of the others, turning on their axes as they do so, so that in the stalk of the sporophyll there is a ring or double row of bundles with their xylems all pointing towards the centre. The ventral bundles of the stalk with inverted orientation nearly all, in the lamina, turn on their axes and become normally orientated, so that two rows of bundles pass upwards through this region; one or two bundles, however, lying somewhat out of the ventral row, towards its inner side, retain their inverted orientation. As the sporophyll was very young, the bundles had not yet attained their full development; but in the stalk, centrifugal and no centripetal xylem was developed.

C. Miquëliana, H. Wendl.

Female Sporophyll. In the lowest part of the stalk there is a row of three or four bundles, of which the two outer lateral ones are of great size, with a very well-developed mass of centrifugal and two or three quite small elements of centripetal xylem. The one or two intermediate bundles are quite small, elongated radially, and may have an element or two of centripetal xylem. In some sporophylls there occur on the ventral side of and lying usually some distance away from

the normal row, several small normally-orientated bundles whose origin, owing to the cone being in a state of decay and the tissue therefore connecting the sporophylls with the axis being partially destroyed, could not be ascertained (Fig. 27). In the case of a small bundle with inverted orientation, lying on the ventral side of one of the large lateral bundles, this was observed to be cut off from this latter, whose phloem gradually extended round the xylem, so as to render the bundle partially concentric in structure, when part of it became severed towards the ventral side, which part, twisting on its axis, formed the small inverted bundle.

In the lamina, the bundles have usually an equal quantity of centrifugal xylem, but in some the latter is absent.

In the young female sporophyll the developing bundles of the lamina appear to *form centrifugal and centripetal xylem at the same time*; in some bundles both kinds are seen together (Fig. 28), in others centripetal alone; in others, again, centrifugal alone; in some there is an intermediate stage where the tracheides lie at the side of the protoxylem. This variation in the respective development of the centrifugal and centripetal xylem coincides with what is found in the mature sporophyll.

In the *barren sporophyll* of this genus the bundles are all of uniform development, and none are so markedly developed as those supplying the sporangia in the fertile sporophyll.

Summary.

The result of my investigations into the structure of the sporophyll for each genus may be thus summarized:—

Cycas, ♂: in lower part of stalk (though probably not at extreme base) centripetal xylem of bundles is equal to, or greater in amount than, centrifugal xylem.

——— ♀: borne directly on vegetative axis; larger and more leaf-like than in any other genus; bundles of large size, with large development of centrifugal and

considerable and constant quantity of centripetal xylem, with small inverted strands on their dorsal side, structure exactly resembling that of bundles in peduncle of *Stangeria*; large strand supplying megasporangium has almost completely concentric structure with few primary tracheides in central pith.

Stangeria, ♂ : two bundles in stalk with purely endarch structure.

——— ♀ : two to four bundles in stalk, of which two lateral ones supplying sporangia are much larger in size; centripetal xylem of two or three tracheides only in one or two bundles.

Bowenia, ♂ : number of very small bundles in stalk, irregularly orientated, and with purely endarch structure.

——— ♀ : four principal bundles in stalk, of which two lateral are largest; few tracheides of centripetal xylem always present in latter and may also occur in smaller bundles; besides these, occur small bundles on ventral side very differently orientated in different sporophylls, and sometimes quite *concentric* in structure.

Dioon, ♂ : number of small bundles in stalk; most have three or four or more centripetal tracheides.

——— ♀ : one of the largest in order, but megasporangia relatively small in size; number of bundles in stalk, but extreme base not represented. Lateral bundles of row more strongly developed than rest only when sporangia they supply are not abortive; small quantity of centripetal xylem present in some bundles.

Encephalartos, ♂ : row of bundles in stalk, of which some are larger than others, and have small amount of centripetal xylem.

——— ♀ : one of the largest in order; bundles in stalk resembling those of ♀ of *Cycas* in structure, but very irregularly orientated and grouped, and more curved in shape, some having almost *concentric*

structure. Centrifugal xylem nearly always present, often in large quantity. Small inverted strands attached to dorsal side of bundles, as in *Cycas*.

Macrozamia, ♂ : two to three bundles in lowest part of stalk, which rapidly divide up into larger number with usual endarch structure; no centripetal xylem.

——— ♀ : one of the largest in order; bundles have very well-developed centrifugal and well-marked quantity of centripetal xylem; bundles resemble very much those in ♀ of *Encephalartos*.

Zamia, ♂ : three to four bundles in stalk with rather irregular arrangement and orientation; centripetal xylem sometimes present; some bundles are partially *concentric* in structure.

Z. Loddigesii, Miq., ♀ : four bundles in stalk, of which two lateral are largest. All bundles have centripetal xylem, whose elements are often larger than those of centrifugal portion.

Z. furfuracea, Ait.; *Z. latifolia*, Lodd.; *Z. Fischeri*, Miq., ♀ : the four bundles have no centripetal xylem. A small bundle, either *concentric* or collateral in structure, occurs on ventral side of row as a branch from one of normal bundles.

Z. Leiboldii, Miq., ♀ : abnormal, bearing *three* sporangia; in consequence, vascular system of stalk is concentrated on side on which two sporangia are borne.

Ceratozamia latifolia, Miq., ♂ : *medullary* bundles, either *concentric* or collateral in structure, in axis of cone; three bundles in stalk of sporophyll; no centripetal xylem.

C. mexicana, Brongn., ♂ : of three bundles in stalk, median one is much smaller than other two.

——— ♀ : ring or double row of bundles in stalk, whose xylem is in all cases directed inwards; these arise from division of two original bundles arising from cylinder of axis. Owing to young state of organ no difference in respective development exists.

C. Miqueliana, H. Wendl., ♀ : row of three to four bundles in stalk, of which two lateral are of great size compared with the others; along with largely developed centrifugal, few small centripetal tracheides usually present, as is also the case with smaller bundles; other smaller bundles, quite apart from normal row, with either normal or inverted orientation.

The more general characters of the sporophyll are the following:—

Male. A single bundle leaves the cylinder of the axis of the cone, which, on entering the stalk of the sporophyll, divides into three The bundles supplying the sporangia are much smaller in size than the similar ones on the female side, owing to the comparatively brief period of attachment to the sporophyll of the microsporangia and the short functional activity of the latter. They also diverge less from the mesarch structure of those of the foliage-leaf than do the bundles of the female sporophyll.

Female. Two bundles leave the cylinder of the axis of the cone, usually dividing up in the cortex into a larger number, so that, as a rule, four bundles occur in the stalk of the sporophyll, of which the two lateral ones are much larger than the rest, this being correlated with their function of supplying the megasporangia during the long period of development and attachment of the latter to the sporophyll. The divergence from the ordinary mesarch structure of the foliage-leaf is much more marked here.

In the *sterile* portion of the sporophyll of both sexes, i. e. the part above the insertion of the sporangia, the mesarch structure of the bundles prevails, showing how, the sporangial element being eliminated, the ordinary and typical structure of foliar bundles reappears. As compared with the bundles of the stalk, the centripetal is as a rule much more developed than the centrifugal xylem, while the phloem, which shares the development of the centrifugal xylem in the stalk, becomes reduced and insignificant.

As a general theory resulting from the preceding investigations, it appears to me a quite probable view that the sporophyll in Cycadaceae is a more primitive foliar organ than the foliage-leaf, and for the following reasons:—

a. The theory of Bower with regard to the development and morphology of spore-producing members in the Lycopodineous series of plant-forms and the phylogenetically late appearance of assimilating foliar appendages, which arise by progressive sterilization of the tissues of the former, appears to me to be a highly natural hypothesis and one containing a large element of truth.

Now, it seems to me highly probable that the Fern-sporophyte had a very similar origin, that it also arose from a fertilized oospore which, instead of producing motile reproductive organs, gradually replaced these latter by asexual, non-motile spores contained in sporangia which eventually became raised into an aerial position by progressive sterilization of the tissues of the young developing sporophyte: a theory which will not be accepted by those who incline to the view that the Fern-sporophyte had an entirely different origin from that of the Bryophytes[1], with which group it is here my purpose to emphasize its similarity of origin. There is, in fact, no reason to think that the order of development of the two kinds of foliar appendages, sporophyll and foliage-leaf, should, in the Fern-series, have been fundamentally different from that in the Lycopodineous series: the ontogenetic developmental history of the two types is to-day so similar as to point to the probability of their phylogenetic developmental history having been also similar.

This being so, we may assume that evolution may have proceeded as follows. First in order of time, sporophytes which bore sporophylls only would exist; next to these would succeed those in which both sporophylls and assimilating foliar organs were present. But it is quite conceivable that subsequent modification of all the sporophylls might

[1] Scott, Address to Botanical Section of Brit. Assoc. Meeting, Liverpool, 1896, pp. 9–10.

have taken place in certain forms, producing types like the Marattiaceae at an early period, so that the sporangia came to be borne on assimilating fronds, as in the case of most modern Leptosporangiate Ferns. On the other hand, the original type, viz., that in which the sporangiferous organs were distinct from the assimilating leaves, probably persisted right through, even down to the present day. From such forms as these latter I imagine it to be quite conceivable, and even probable, that modern Cycads took their origin.

b. From the investigations of Solms-Laubach, Scott, and myself, it appears that the *peduncle* of these plants has in several ways, notably in the simplified vascular bundle-system, the presence of a mesarch structure in the bundles of the central cylinder, and of concentric strands in various parts of the organ, a more primitive structure than the vegetative stem, and probably therefore more nearly represents the original typical stem-structure. This being the case, it might naturally be expected that the foliar appendages of the peduncle would possess a more primitive structure than the foliar appendages of the vegetative stem.

c. This primitive structure is represented by the *concentric* bundles which occur in both the fertile and barren sporophylls of several genera, especially in the latter organs, where the special physiological function of the former has not interfered with the original structure. These concentric bundles are absent from the foliage-leaves, the structure, number, and orientation of whose bundles are extremely regular, constant, and well-defined, whereas in the sporophylls the reverse is the case, a fact which probably points to their possessing a more primitive structure, viz., one not so perfectly adapted and stereotyped to subserve a special physiological function, as is the case with the foliage-leaves.

In conclusion, the special point upon which I desire to lay stress in this paper is this: that though the sporophyll, according to my view, is a more primitive organ than the foliage-leaf, for the reasons above adduced, the main and, physiologically, most important part of its vascular structure

has become, as a result of the sporangiferous function, much more highly modified from the primitive type than that of the foliage-leaves, this special function influencing more or less all parts of the vascular system. But though this modification in structure has taken place, it has not obscured, to such an extent as in the foliage-leaves, the primitive character of the organ which, either in the form of concentric or partially concentric strands, of irregular orientation and arrangement of the bundles, of great variability in size of the latter, or of a tendency to abortion of certain of the more primitive parts of the vascular system, appears over and over again, in one place or another, throughout all the genera investigated.

I desire to express my obligation to Dr. D. H. Scott for all the assistance and the numerous criticisms which he has, as usual, so kindly afforded me.

EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Mr. Worsdell's paper on the Cycadaceae.

The following are the abbreviations used:—*ph.* phloem; *ph*^{1}. ventral phloem; *x*^{2}. centrifugal xylem; *x*^{1}. centripetal xylem; *px.* protoxylem; *tf.* transfusion-tissue; *pb.* bundle from peduncle; *fb.* foliar bundle; *ped.* peduncle; *bs.* barren sporophyll; *sph.* sporophyll (fertile); *sp.* sporangium; *lm.* lamina; *st.* stalk.

Fig. 1. *Cycas circinalis*, L. Diagrammatic transverse view of the arrangement of the vascular bundles in the stalk of the ♀ sporophyll. × 35.

Fig. 2. *Cycas circinalis*, L. Transverse section of a strand from the stalk of the ♀ sporophyll. × 65.

Fig. 3. *Cycas circinalis*, L. Transverse section of a bundle from the lamina of the ♀ sporophyll. × 100.

Fig. 4. *Cycas revoluta*, Thunb. Transverse section of a concentric strand about to enter the megasporangium. × 65.

Fig. 5. *Stangeria paradoxa*, Th. Moore. Diagram of the vascular bundle-system of the ♀ sporophyll.

Fig. 6. *Stangeria paradoxa*, Th. Moore. Diagrammatic transverse view of the arrangement of the vascular bundles in the stalk of the ♀ sporophyll. × 35.

Fig. 7. *Stangeria paradoxa*, Th. Moore. Transverse section of one of the bundles in the stalk of the ♀ sporophyll which supply the megasporangia. × 145.

Fig. 8. *Stangeria paradoxa*, Th. Moore. Transverse section of a bundle from the lamina of the ♀ sporophyll. × 145.

Fig. 9. *Stangeria paradoxa*, Th. Moore. Diagram of the vascular bundle-system of a barren sporophyll at base of ♂ cone.

Fig. 10. *Stangeria paradoxa*, Th. Moore. Transverse section of a concentric bundle in a barren sporophyll. × 300.

Fig. 11. *Bowenia spectabilis*, Hook. Diagrammatic transverse view of the arrangement of the vascular bundles in the stalk of the ♀ sporophyll. × 35.

Fig. 12. *Bowenia spectabilis*, Hook. Transverse section of a concentric bundle from stalk of the ♀ sporophyll. × 450.

Fig. 13. *Bowenia spectabilis*, Hook. Basal part of young ♂ cone, showing the barren sporophylls. Nat. size.

Fig. 14. *Bowenia spectabilis*, Hook. Transverse section of a concentric bundle in a barren sporophyll. × 450.

Fig. 15. *Dioon edule*, Lindl. Transverse section of one of the bundles which supply the megasporangia in the stalk of the ♀ sporophyll. × 100.

Fig. 16. *Dioon edule*, Lindl. Transverse section of a bundle in the lamina of the ♀ sporophyll. × 225.

Fig. 17. *Dioon edule*, Lindl. Diagram of the vascular bundle-system supplying the megasporangium.

Fig. 18. *Encephalartos horridus*, Lehm. Transverse section of a partially concentric bundle in the stalk of the ♀ sporophyll. × 110.

Fig. 19 *Encephalartos horridus*, Lehm. Transverse section of a bundle in the stalk of the ♀ sporophyll. × 110.

Fig. 20. *Encephalartos horridus*, Lehm. Transverse section of a concentric bundle entering the megasporangium (semi-diagrammatic). × 100.

Fig. 21. *Zamia Lindeni*, Regel. Diagrammatic transverse view of the arrangement of the bundles in the stalk of the ♂ sporophyll. × 50.

Fig. 22. *Zamia Lindeni*, Regel. Diagrammatic transverse view of the arrangement of the bundles in the stalk of the barren sporophyll. × 50.

Fig. 23. *Zamia Loddigesii*, Miq. Transverse section of a bundle in the lamina of the ♀ sporophyll. × 145.

Fig. 24. *Zamia Loddigesii*, Miq. Transverse section of a bundle supplying a barren sporophyll from the peduncle of the ♀ plant. × 100.

Fig. 25. *Zamia Leiboldii*, Miq. Dorsal and lateral views of an anomalous ♀ sporophyll, bearing three sporangia. Nat. size.

Fig. 26. *Ceratozamia mexicana*, Brongn. Diagrammatic transverse view of the arrangement of the bundles in the stalk of the ♂ sporophyll. × 35.

Fig. 27. *Ceratozamia Miqueliana*, H. Wendl. Diagrammatic transverse view of the arrangement of the bundles in the stalk of the ♀ sporophyll. × 20.

Fig. 28. *Ceratozamia Miqueliana*, H. Wendl. Transverse section of a bundle in the lamina of a young ♀ sporophyll. × 450.

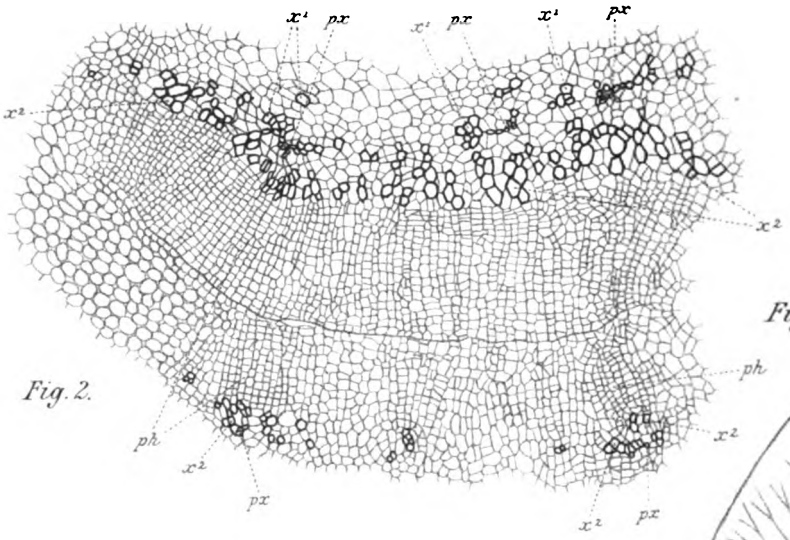


Fig. 2.

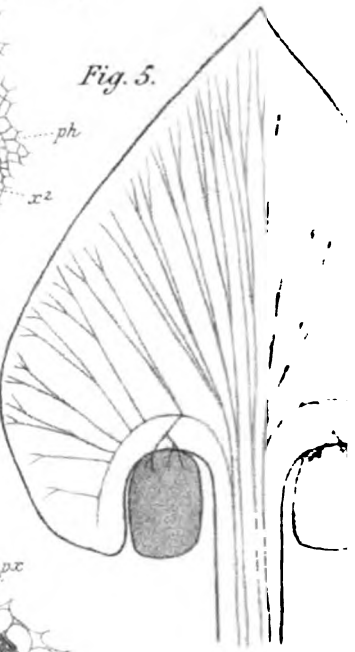


Fig. 5.

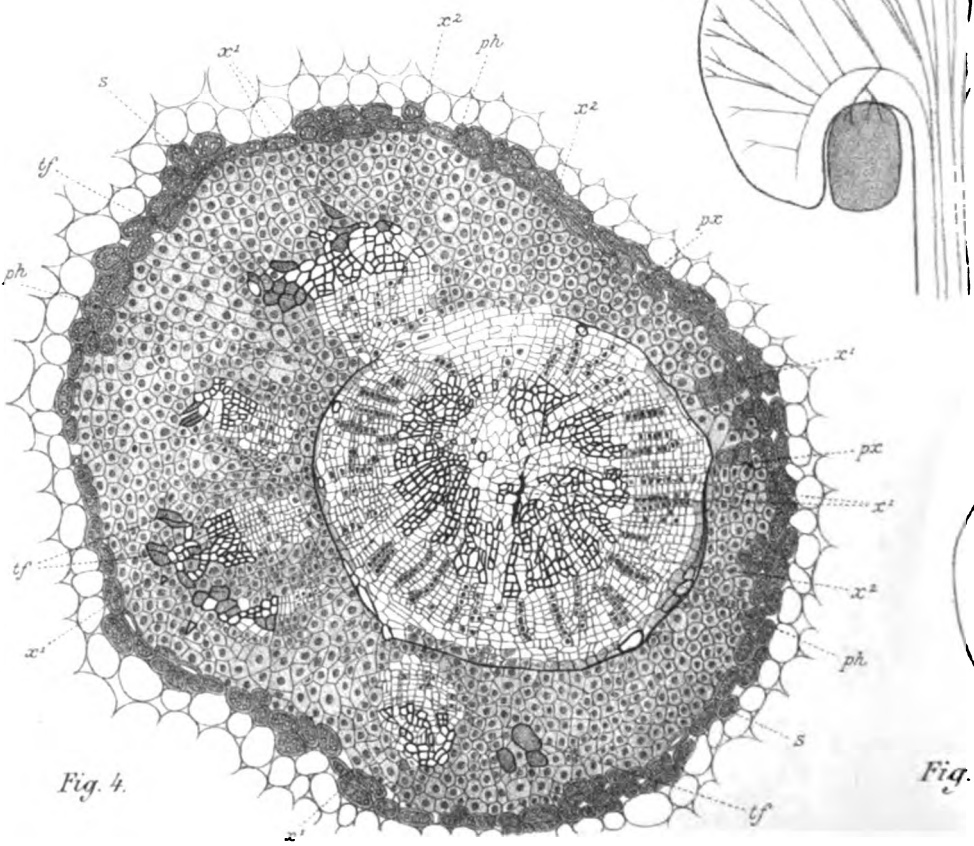


Fig. 4.

Fig. II.

W. C. Worsdell. del.

WORSDELL.— SPOROPHYLLS OF CYCADACEAE.

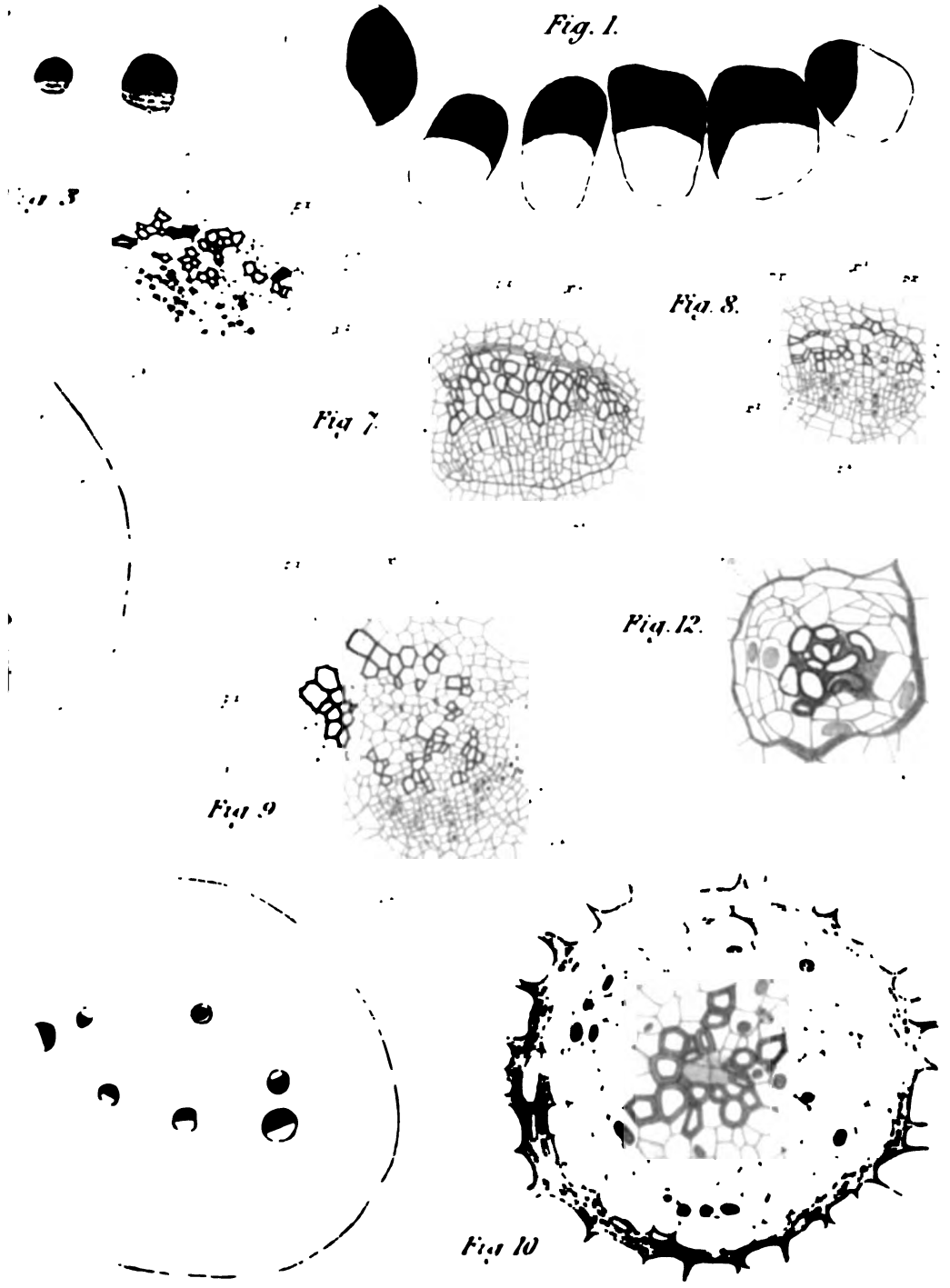


Fig. 1.
Fig. 7.
Fig. 8.
Fig. 9
Fig. 10
Fig. 12.

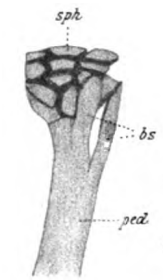


Fig. 13.

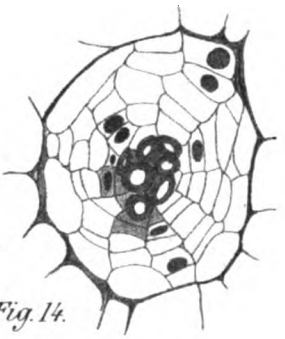


Fig. 14.

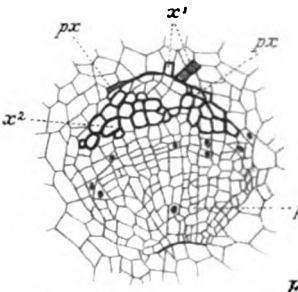


Fig. 15.

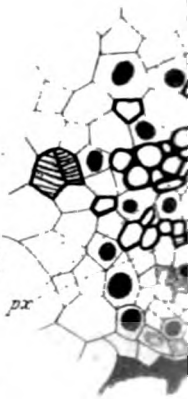


Fig. 16.

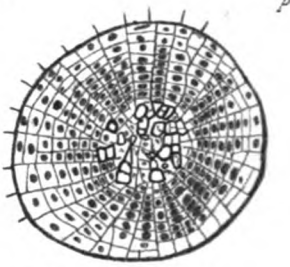


Fig. 20.

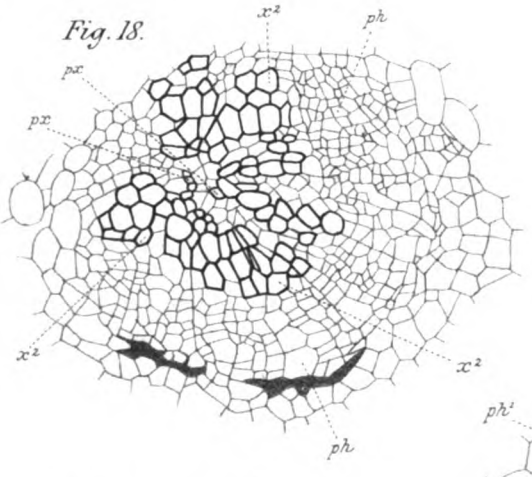


Fig. 18.

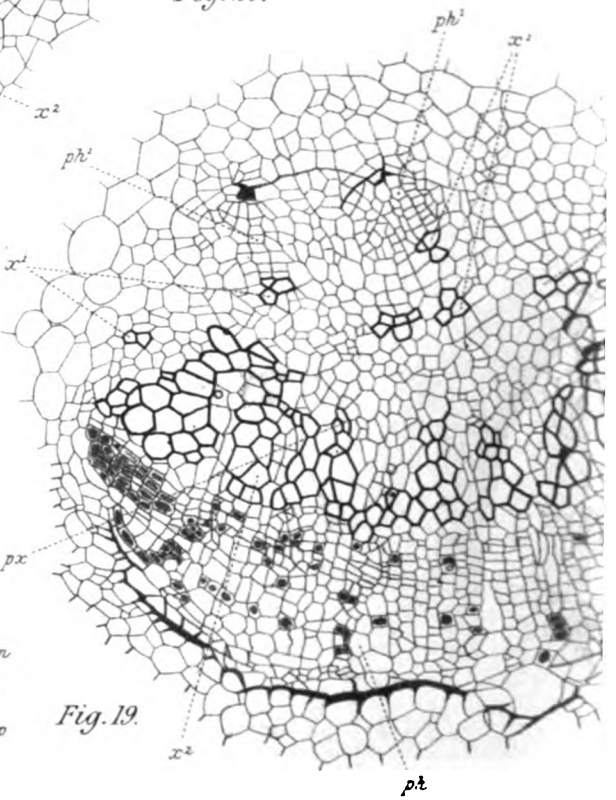


Fig. 19.

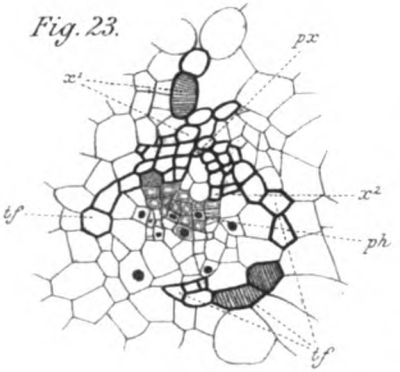


Fig. 23.

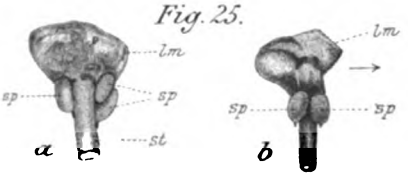
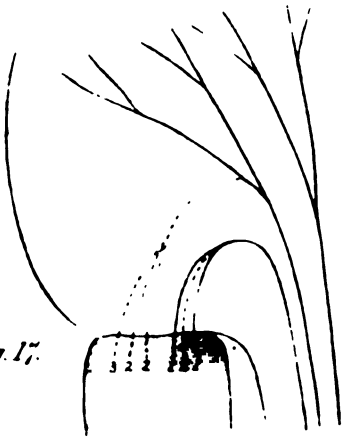


Fig. 25.

W. C. Worsdell, del.

WORSDELL.— SPOROPHYLLS OF CYCADACEAE.

Fig. 17.



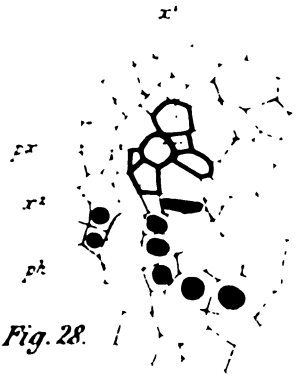


Fig. 28.

Fig. 21

Fig. 22

Fig. 26

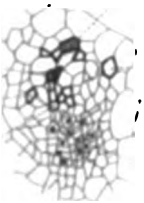


Fig. 24

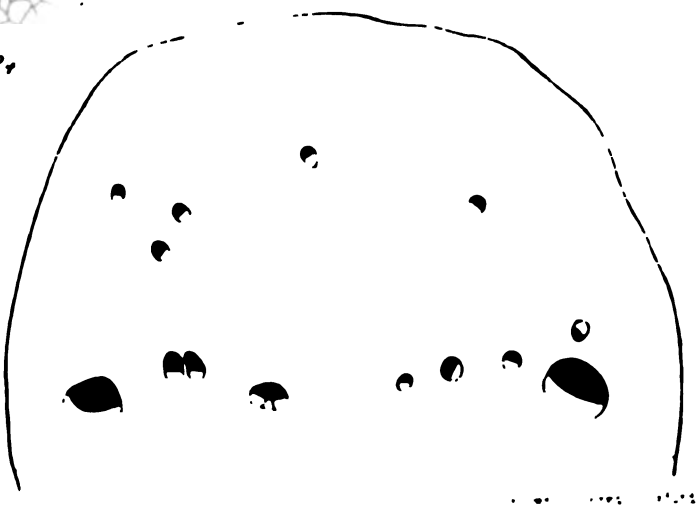


Fig. 27

Further Contributions to the Geological History of the British Flora.

BY

CLEMENT REID, F.L.S., F.G.S.

THE past ten years have yielded much information which was not available when my former paper was written[1]. They have witnessed so great an accumulation of evidence relating to the origin of the British flora, to the climatic changes which expelled or brought back this flora, and to the means of dispersal by which our plants were able to regain their lost position, that the subject is no longer within the compass of an article in a scientific journal. I am compelled, therefore, to reserve all details for publication in book-form; but as the volume cannot be completed for another year, I have in this paper tabulated some of the leading results.

Before entering into details as to the range in time of our British plants, it may be useful in a few words to summarize the results now arrived at. This will be done without touching more than is necessary on the various debatable conclusions to which the researches seem to lead. The notes

[1] Annals of Botany, Vol. II, p. 177. No. VI, Aug. 1888.

shall be confined as far as possible to the facts, and to such inferences as to former climatic changes as seem to follow necessarily from a study of the botanical evidence. Without some idea of the prehistoric state of Britain the records would be of little value. I doubt whether many, even of the geologists, have realized that ceaseless ebb and flow which prevents our fauna and flora from exhibiting more than the rudest adaptation to the present state of Britain. Possibly in some part of the Tropics a balance may have been arrived at; but in Britain the last climatic changes have taken place at too recent a date for such a result, and the composition of our flora is still undergoing constant modification.

About one-seventh of our flowering plants have now been found in the fossil state. This is by no means a large proportion; yet considering that fully a third of our species have neither deciduous leaves, woody stems, nor hard seeds, and are unlikely therefore to be often preserved as fossils, it is not unsatisfactory. The orders are very unequally represented. In the first place, annual plants with soft seeds are almost entirely absent. Orders such as the Cruciferae and Orchideae are wanting; Grasses are found, but only as indeterminable nodes or leaves. These deficiencies are easy to understand; but the absence of Leguminosae and of the aquatic species of *Veronica* and *Scrophularia* is less comprehensible, and makes one speculate as to whether the deficiency is real, or in part due to the more perishable nature of the pods and seeds, though these may seem quite hard enough to be preserved. The orders best represented are mainly those which possess hard fruits or seeds specially adapted for dispersal, and those with deciduous leaves. Ranunculaceae, Caryophyllaceae, Rosaceae, Cupuliferae, Naiadaceae and Cyperaceae are all fairly well represented; Umbelliferae are not uncommon, though seldom in a determinable state. Fruits of Compositae are characteristic and do not readily decay; various species belonging to this order have been discovered, but they are usually represented by a few wind-borne specimens. The order is likely to yield

an exceptionally complete record; its entire absence from the older Tertiary deposits is therefore all the more striking.

The deposits from which existing species of plants have been obtained in Northern Europe may be grouped roughly into four series; but as the number of cold or warm waves that have passed since Pliocene times is still uncertain, the classification adopted is provisional, and may need to be considerably extended. The provisional grouping used in this paper is as follows:—

Neolithic:—Post-Glacial, but Pre-Roman. 'Submerged forests,' and alluvial or lacustrine deposits with a Temperate flora. Cultivated plants and weeds of cultivation appear. (Part of the Scottish plant-bearing strata may be of more recent date.)

Late Glacial:—Lacustrine deposits with Arctic plants above the latest deposits showing ice-action.

Interglacial:—Deposits with Temperate plants, between strata indicating Arctic conditions.

Early Glacial:—Flood-loams with Arctic plants, at the base of the Glacial deposits of Norfolk.

Preglacial:—Newest Pliocene deposits (Cromer Forest-bed).

A word of warning is perhaps needed as to the use of the table for the determination of species as 'native' or 'introduced.' The term native though convenient is misleading, for it is doubtful whether a single one of our flowering plants is really native of Britain. The whole flora has originated probably in other and various parts of the world. We find now merely the species stranded by successive waves of migration, which have brought together a variety of continental forms, some Arctic, some Southern, a few even American. These migrations were mainly compelled by climatic changes, though other agencies have played an important part. To judge by the evidence already obtained, though negative evidence does not go for much, it seems probable that a far larger proportion of our plants was introduced by human agency than has been thought.

TABLE SHOWING THE GEOLOGICAL RANGE OF THE RECENT BRITISH FLORA.

E = England
W = Wales
S = Scotland
M = Isle of Man
I = Ireland
D = Denmark
F = Finland
G = North Germany
N = Norway
Sw = Sweden

} only recorded where the species is unknown from homotaxial deposits in Britain.

	Preglacial.	Early Glacial.	Interglacial.	Late Glacial.	Neolithic.
Clematis Vitalba, L.	...	...	E ?		
Thalictrum minus, L.	...	E	...	S	
,, *flavum*, L.	E	...	E	...	Sw
Ranunculus aquatilis, L.	E	E	E S	E S	E S I M
,, *sceleratus*, L.	...	...	E	E	E
,, *Flammula*, L.	...	...	E	E	S M
,, *Lingua*, L.	...	...	E	...	S
,, *repens*, L.	E	...	E	E S	E S M
,, *bulbosus*, L.	...	...	E		
,, *Sardous*, Crantz	...	...	E		
,, *parviflorus*, L.	...	...	E		
Caltha palustris, L.	E	...	E	E	S
Nuphar luteum, Sm.	E	...	E	...	E
Nymphaea alba, L.	...	...	G	...	Sw
Papaver somniferum, L.	...	...	...	...	S
Fumaria officinalis, L.	...	...	...	...	S
Cakile maritima, Scop.	...	...	...	...	Sw
Viola palustris, L.	E	...	E S	E S	S M W
Silene maritima, L.	...	...	E		
Lychnis alba, *Mill.*	...	...	...	...	S
,, *diurna*, Sibth.	...	...	...	S	S
,, *Flosculi*, L.	...	...	...	S	S
Stellaria aquatica, Scop.	E				
,, *media*, *Cyr.*	E	...	E	E S	S
,, *uliginosa*, L.	...	...	..	...	S
Arenaria trinervia, L.	...	...	G		
Spergula arvensis, L.	...	...	...	...	S
Montia fontana, L.	...	...	E	E	S
Hypericum quadrangulum, L.	...	...	...	...	S
,, *elodes*, L.	...	...	...	...	S
Tilia platyphyllos, Scop.	..	...	G	...	Sw
Linum, *sp.*	...	...	...	...	S
Geranium columbinum, L.	...	...	G		
Oxalis Acetosella, L.	...	...	...	S	E S
Ilex Aquifolium, L.	...	...	G	...	E
Rhamnus Frangula, L.	...	...	E	...	Sw

	Preglacial.	Early Glacial.	Interglacial.	Late Glacial.	Neolithic.
Acer campestre, L.	E	...	G		
,, *monspessulanum*, L.	...	...	E		
Prunus communis, Huds.	E	...	E	...	E S
,, *domestica*?, L.	...	...	...	...	E S
,, *Avium*, L.	...	...	E	...	E S
,, *Padus*, L.	...	...	E S	...	E S
Spiræa Ulmaria, L.	...	...	E	...	S
Rubus Idæus, L.	...	E	E	E S	E S
,, *fruticosus*, L.	E	...	E	...	E S M
,, *cæsius*, L.	...	...	...	...	Sw
,, *nemorosus*, L.	...	...	...	...	Sw
Dryas octopetala, L.	...	...	...	S	
Potentilla Tormentilla, Neck.	...	...	E	S ?	S M
,, *Comarum*, Nestl.	...	...	S	E	S M
Alchemilla arvensis, L.	...	...	...	...	S
Poterium officinale, Hook.	E	E	E	E S	S M
Rosa canina, L.	...	...	E	...	S
Pyrus terminalis, Ehrh.	...	...	E		
,, *Aucuparia*, Gaert.	...	...	...	...	W
,, *communis*, L.	...	...	...	...	E
Crataegus Oxyacantha, L.	E	...	E	...	E W S
Saxifraga oppositifolia, L.	...	...	...	G	
,, *Hirculus*, L.	...	...	...	G	
,, *azoides*, L.	...	...	...	G	
Hippuris vulgaris, L.	E	E	E S	E S	E S M
Myriophyllum spicatum L.	E	E	E S	E S	S I M
,, *alterniflorum*, L.	...	...	...	...	Sw
Trapa natans, L.	E	...	G	...	Sw F
Hydrocotyle vulgaris, L.	E	...	E	..	S M
Apium nodiflorum, Reich.	...	...	S		
Cicuta virosa, L.	...	...	..	...	Sw
Sium latifolium, L.	...	...	...	...	Sw
Œnanthe Lachenalii, Gmel.	E	...	...	S	
,, *crocata*, L.	...	...	E ?		
,, *Phellandrium*, Lam.	...	...	E	E	E
Æthusa Cynapium, L.	.	...	...	..	S
Angelica sylvestris L.	...	...	E	...	Sw
Peucedanum palustre, Moench.	..	...	..	...	Sw
Heracleum Sphondylium, L.	E	...	...	E	
Hedera Helix L.	..	...	E	...	E W
Cornus mascula, L.	...	..	...	Sw	
,, *sanguinea*, L.	E	...	E	...	E W
Sambucus nigra, L.	.	...	E	E	E S
Viburnum Opulus, L.	..		E	.	S
Galium boreale, L.	..	E?			
,, *palustre*, L.	...	...	G	.	S ?
,, *uliginosum*, L		.	G		
,, *Aparine*, L.					E ?
Valeriana officinalis, L.		...	F		W S
Scabiosa succisa, L.	...	..	F		
Eupatorium cannabinum, L		..	E	F	S

	Preglacial.	Early Glacial.	Interglacial.	Late Glacial.	Neolithic.
Bidens cernua, L.	...	...	...	...	S
,, *tripartita*, L.	...	...	E	E	
Chrysanthemum segetum, L.	...	...	...	...	S
Matricaria inodora, L.	...	...	...	...	S
Tanacetum vulgare, L.	...	E			
Tussilago Farfara, L.	...	...	...	...	S
Senecio sylvaticus, L.	...	...	...	...	S
Carduus crispus, L.	...	...	...	...	M
,, *lanceolatus*, L.	...	...	E	...	S
,, *palustris*, L.	...	...	E	...	S
Centaurea Cyanus, L.	...	...	...	...	S
Lapsana communis, L.	E	...	E	...	S
Picris hieracioides, L.	E				
Crepis virens, L.	...	...	...	...	S
Leontodon autumnalis, L.	...	...	E	...	S
Taraxacum officinale, Web.	...	...	E	E S	S
Sonchus arvensis, L.	...	...	...	...	S
Vaccinium Oxycoccos, L.	...	...	G	...	Sw
,, *Vitis-Idaea*, L.	...	...	...	G	Sw
,, *uliginosum*, L.	...	...	G	Sw	Sw
,, *Myrtillus*, L.	...	...	...	G	
Arctostaphylos alpina, Spreng.	...	...	...	Sw	
,, *Uva-ursi*, Spreng.	...	...	...	E	Sw
Andromeda Polifolia, L.	...	...	...	S	
Loiseleuria procumbens, Desv.	...	...	...	S	
Fraxinus excelsior, L.	...	...	E	...	E
Menyanthes trifoliata, L.	E	E	E S	E S	W S M
Myosotis sylvatica, Hoffm.	...	...	...	...	Sw
Solanum Dulcamara, L.	...	...	...	...	Sw
Bartsia Odontites, Huds.	...	...	...	S	
Pedicularis palustris, L.	...	...	...	...	S
Mentha aquatica, L.	...	...	E		
Lycopus europaeus, L.	E	...	E	E	S
Thymus Serpyllum, L.	...	...	...	S	
Prunella vulgaris, L.	...	...	...	...	S
Stachys palustris, L.	E	...	E ?	S	S
,, *sylvatica*, L.	...	...	...	Sw	Sw
Galeopsis Tetrahit, L.	...	...	...	E	S
Ajuga reptans, L.	...	...	E	E S	S
Littorella lacustris, L.	...	...	...	M	S ?
Atriplex patula, L.	E	...	E	S	E S
Polygonum aviculare, L.	...	...	E	S	S
,, *Hydropiper*, L.	...	...	...	...	E
,, *Persicaria*, L.	...	...	E	E	S
,, *lapathifolium*, L.	...	...	...	...	S ?
,, *amphibium*, L.	...	...	...	...	Sw
,, *viviparum*, L.	...	...	...	G Sw	
Oxyria digyna, Hill.	...	...	...	S	
Rumex conglomeratus, Murr.	...	...	E		
,, *maritimus*, L.	E	E	E	E	
,, *obtusifolius*, L.	...	...	E	...	S M

	Preglacial.	Early Glacial.	Interglacial.	Late Glacial.	Neolithic.
Rumex crispus, L.	E	...	E	E S	E W S
,, *Hydrolapathum*, Huds	...	...	...	...	Sw
,, *Acetosella*, L.	E	...	E		
Hippophae rhamnoides, L.	...	...	...	...	Sw
Viscum album L.	...	...	...	...	Sw
Euphorbia Helioscopia, L.	...	...	...	...	S
,, *amygdaloides*, L.	E				
Mercurialis perennis, L.	...	...	E	...	E S
Ulmus montana?, Sm	E	...	E	...	E
Urtica dioica, L.	...	...	E		
Myrica Gale, L.	...	...	G	...	Sw
Betula alba, L	E	...	...	E S	E W S I
,, *nana*, L.	...	E	E S	E S	
Alnus glutinosa, L.	E	E	E S	E S	E S
Carpinus Betulus, L.	E	...	E		
Corylus Avellana, L.	E	...	E S	...	E W S
Quercus Robur, L.	E	...	E	...	E W S
Castanea sativa, Mill.	...	...	E ?		
Fagus sylvatica, L.		...	G	E	
Salix pentandra, L.	...	...	G ?	...	Sw
,, *cinerea*, L.	E	...	G	E	Sw
,, *aurita*, L.	...	...	G	...	Sw
,, *Caprea*, L.	...	...	G	...	E W
,, *phylicifolia*, L.	...	...	...	Sw	Sw
,, *nigricans*, Sm.	...	...	...	...	Sw
,, *repens*, L.	...	...	G	S	E S
,, *lanata*, L.	...	...	...	...	Sw
,, *Arbuscula*, L.	...	...	...	G ?	Sw
,, *Myrsinites*, L.	...	...	...	E	
,, *herbacea*, L.	...	...	S	E S M	
,, *polaris*, Wahlb.	...	E	...	E S	
,, *reticulata*, L.	...	...	...	S	
Populus canescens, Sm.	...	...	E ?		
,, *tremula*, L.	...	...	G	...	W
Empetrum nigrum, L.	...	...	S	S M	
Ceratophyllum demersum, L.	E	E	E	E	E
Juniperus communis, L.	...	...	G	Sw	Sw
Taxus baccata, L.	E	...	E	...	E S
Picea excelsa, Link.	E	...	G	...	Sw
Pinus sylvestris, L.	E	...	...	E	E S I
Stratiotes aloides, L.	E	...	E		
Iris Pseudacorus, L.	...	...	...	...	E S
Sparganium ramosum, Curtis	E	...	E	E S	E W S
,, *simplex*, Huds.	...	...	G		
,, *minimum*, Fr.	...	...	G		
Alisma Plantago, L.	E	...	E	E	S
Sagittaria sagittifolia, L.	...	...	...	...	Sw
Scheuchzeria palustris, L.	...	...	...	Sw	Sw
Potamogeton natans, L.	...	..	E	...	W
,, *rufescens*, Schrad	...	...	S	E	
,, *heterophyllus*, Schreb	E	...	E S	...	W S

	Preglacial.	Early Glacial.	Interglacial.	Late Glacial.	Neolithic.
Potamogeton lucens, L.	E	...	...	...	S
,, *praelongus*, Wulf.	...	...	G	Sw	I
,, *perfoliatus*, L.	E	...	E	...	S
,, *crispus*, L.	E	...	E	E	S I M
,, *obtusifolius*, M. & K.	...	...	E		
,, *pusillus*, L.	...	...	E	E	S
,, *trichoides*, Cham.	E	...	E	E	
,, *pectinatus*, L.	E	...	...	E	S
,, *filiformis*, Nolte.	...	...	...	Sw	Sw
Ruppia maritima, L.	...	...	E	...	Sw
Zannichellia palustris, L.	E	E	E S	E	
Zostera marina, L.	...	...	G	...	Sw ?
Najas flexilis, Rostkov.	...	...	...	...	Sw
,, *marina*, L.	E	...	E	...	W
Eleocharis acicularis, Sm.	...	...	E		
,, *palustris*, Br.	...	E	E	E S	S I M
Scirpus pauciflorus, Lightf.	E	...	E	E S	S
,, *caespitosus*, L.	E	...	E		
,, *fluitans*, L.	E	...	E	S	
,, *setaceus*, L.	...	...	E	E	S
,, *lacustris*, L.	E	E	E	E S	E S
,, *maritimus*, L.	...	...	...	...	E W
,, *sylvaticus*, L.	...	...	...	...	Sw
Blysmus rufus, Wahlb.	...	...	E	E	
Eriophorum vaginatum, L.	...	...	G	...	Sw
,, *angustifolium*, Roth.	E	...	E	...	Sw
Cladium Mariscus, Br.	...	...	G	...	Sw
Carex dioica, L.	...	...	E S	S	S
,, *echinata*, Murr.	...	...	G	...	S
,, *remota*, L.	E				
,, *alpina*, Sw.	...	...	...	M	
,, *canescens*, L.	...	...	...	...	S
,, *panicea*, L.	...	...	S	E	S
,, *distans*, L.	...	...	E		
,, *flava*, L.	...	...	...	...	S
,, *filiformis*, L.	...	...	...	...	Sw
,, *Pseudo-cyperus*, L.	...	...	G	...	Sw
,, *paludosa*, Good.	E				
,, *riparia*, Curtis	E	...	E	...	Sw
,, *rostrata*, Stokes	...	...	E S	...	S ?
,, *vesicaria*, L.	...	...	...	...	Sw
Phragmites communis, Trin.	E	...	E	...	E W
Pteris aquilina, L.	...	...	...	...	Sw
Athyrium Filix-foemina, Roth.	...	...	...	...	Sw
Scolopendrium vulgare, Sm.	...	...	...	...	E ?
Lastraea Thelypteris, Presl.	...	...	G	...	Sw
Osmunda regalis, L.	E	...	...	...	E
Equisetum palustre, L.	...	...	G		
,, *limosum*, Sm.	...	...	G		
,, *hyemale*, L.	...	...	...	...	Sw
Isoetes lacustris, L.	E ?	E	E S	S	S

NOTES.

ON APOGAMY AND THE DEVELOPMENT OF SPORANGIA UPON FERN-PROTHALLI. By WILLIAM H. LANG, M.B., B.Sc.[1]—The two most important deviations from the normal life-history of Ferns, apogamy and apospory, are of interest in themselves, but acquire a more general importance from the possibility that their study may throw light on the nature of alternation of generations in archegoniate plants. They have been considered from this point of view by Pringsheim, and by those who, following him, regard the two generations as homologous with one another in the sense that the sporophyte arose by the gradual modification of individuals originally resembling the sexual plant. Celakovsky and Bower, on the other hand, maintain the view that the sporophyte, as an interpolated stage in the life-history arising by elaboration of the zygote, is not the homologue of the gametophyte, and is only represented in a few Thallophytes. In the light of the theory of antithetic alternation no weight is attached to apogamy and apospory for phylogenetic purposes.

In the paper of which this is an abstract, the results obtained by cultivating the prothalli of a number of species of Ferns under conditions slightly different from the natural ones are described, and their bearing on the problem of the nature of alternation considered. The behaviour of *Scolopendrium vulgare*, Sm., and *Nephrodium dilatum*, Desv., in which sporangia were borne upon the prothallus, has already been described in a preliminary statement[2]. It is therefore sufficient to express the results of prolonged cultivation of these and the remaining species in a tabular form.

[1] Abstract of a paper read before the Royal Society, March 3, 1898.

[2] Roy. Soc. Proc., Vol. lx, p. 250.

TABLE of the Results of cultivating Prothalli for a period of Two Years and a-half.

[*Note.*—In every species normal embryos were produced when conditions permitted fertilization.]

Names.	*Results.*
Scolopendrium vulgare, Sm., var. *ramulosissimum.*	Gametophytic budding. Development of archegonial projections. Development of cylindrical process usually from the apical region of the prothallus. Apogamy. { Tracheides in cylindrical process. / Leaves, roots, and ramenta on process. / Sporangia on the process. / Vegetative buds from tip of cylindrical process, or in place of an archegonial projection.
var. *marginale.*	Similar to var. *ramulosissimum*, but no sporangia, isolated ramenta, or leaves found.
Nephrodium dilatatum, Desv., var. *cristatum gracile.*	Gametophytic budding. Development of archegonial projections. Development of cylindrical process, usually from the under-surface just behind the apex, which formed a 'middle lobe.' Apogamy. { Tracheides in middle lobe and cylindrical process. / Sporangia, sometimes associated with ramenta, on middle lobe and process. / No vegetative buds.
Nephrodium Oreopteris, Desv., var. *corvnans.*	Gametophytic budding. Development of archegonial projections. Development of cylindrical process from apex of prothallus. Apogamy. { Tracheides in cylindrical process. / Ramenta on cylindrical process. / Vegetative buds (rare).
Aspidium aculeatum, Sw., var. *multifidum.*	Gametophytic budding. Development of archegonial projections. Apogamy. { Tracheides in prothallus. / Vegetative buds (rare).

Names.	*Results.*
Aspidium angulare, Willd., var. *foliosum multifidum*.	Gametophytic budding. Development of archegonial projections. Apogamy. { Ramenta on prothallus. Vegetative buds (frequent).
var. *acutifolium multifidum*.	Gametophytic budding. Development of archegonial projections. No apogamy seen.
Athyrium niponicum, Mett., normal form.	Gametophytic budding. Development of archegonial projections. Apogamy. { Tracheides in prothalloid growths from archegonial projections.
var *cristatum*.	Similar to the normal form, but in addition a few apogamously-produced vegetative buds.
Athyrium Filix-fœmina, Bernh.	Gametophytic budding.
var. *percristatum*.	Development of archegonial projections.
var. *cruciatum cristatum*.	Development of cylindrical process from apex
var. *coronatum*.	or from under-surface of the prothallus.
	Apogamy. { Tracheides in process. Continuation of process as a leaf. Vegetative buds.
Polypodium vulgare, L., var. *grandiceps*.	Gametophytic budding. Apogamy. { Isolated leaf-like growths. Vegetative buds (numerous).
Aspidium frondosum, Lowe (from the Pits, Royal Gardens, Kew).	Apogamy. Vegetative buds produced on short cylindrical processes before the culture had been watered. After the culture was watered, normal embryos.

In addition to the species mentioned in the Table above, cultures were made of crested and uncrested forms of *Nephrodium Filix-mas*, Rich., representing the three sub-species, which are sometimes distinguished in this country. Some of these (both crested and normal) behaved in a similar manner to the species referred to in the Table, though only one instance of apogamy induced by long cultivation has as yet been found. Others (crested and normal forms) produced a single bud on the under-side of the prothallus which did not bear archegonia.

Connecting this latter type of apogamy, which agrees with the description of De Bary and Kny, with the more normal prothalli, was one variety, the archegonia of which developed into typical arche-

gonial projections. In the place of the projection nearest to the apex a vegetative bud arose.

It is possible to draw some general conclusions from this series of cultures. It is a striking fact that in every one of the species, prothalli, which under normal conditions would have produced normal embryos, became, after a longer or shorter period, apogamous. Further, there was a general similarity in the changes of form and structure of the prothallus, which preceded this result. This form of apogamy, occurring after prolonged cultivation of normal prothalli under special conditions, may be distinguished as *induced apogamy*, in contradistinction to *direct apogamy*, by which is meant the immediate production of vegetative buds by prothalli, which are usually incapable of being fertilized. Both forms occur in *Nephrodium Filix-mas*.

The causes which appeared to induce apogamy in these prothalli were the prevention of contact with fluid water which rendered fertilization impossible, and the exposure to direct sunlight. Possibly the temperature also had some effect. The case of *Nephrodium Filix-mas* shows that the variable condition of the sporophyte, as indicated by cresting, &c., though possibly predisposing to the changes which lead to apogamy, does not stand in any necessary connexion with the phenomenon.

That different degrees of apogamy are distinguishable was also shown by these cultures. The cylindrical process, arising from the apex of the prothallus, or from its under-surface, is to be regarded simply as a modification in form and structure of the gametophyte dependent on the altered conditions, and possibly a direct adaptation to these. The next stage is seen in cylindrical processes, which, while bearing sexual organs, also produce isolated members of a sporophyte (roots, ramenta, sporangia). It is to be borne in mind, however, that tissue differing from the rest of the process always occurred beneath the last-named structures. The final stage is the production of a vegetative bud capable of further growth as a typical sporophyte. In this a series leading from the bud arising by transformation of the tip of a cylindrical process, to buds produced on or in the place of archegonial projections, and from this to buds situated on the under-surface of the prothallus itself, can be recognized.

The readiness with which the intermediate form between gameto-

phyte and sporophyte and the early stages of vegetative buds re-assume the prothalloid form is worthy of note, as bearing on some cases of apospory.

These departures from the normal development of the prothallus are not regarded as reversions in the ordinary sense, but as indications of the capability of direct response to altered conditions possessed by the gametophyte. Their possible importance in relation to the theory of homologous alternation appears to the writer to be of this nature. If that theory be true, the sporophyte and gametophyte are modifications of a similar form. The gametophyte, especially the simple free living prothallus of the Ferns, has departed less widely from that form. Such an organism as a fern-prothallus would therefore appear to be suitable for experimental work, in the hope that its behaviour under altered conditions would afford hints as to the sort of changes which, in the original algal form, led to the evolution of the sporophyte. The altered conditions in this series of experiments are of a similar kind to those which are assumed by Professor Bower to have occurred on the spread of algal forms to the land, and to have conduced to antithetic alternation.

The results may now be used in picturing the manner in which alternation of generations might have come about by the modification of originally similar individuals into gametophyte and sporophyte. It is assumed for this purpose that the sporophyte of the Vascular Cryptogams did not arise by the elaboration of a structure resembling a bryophytic sporogonium. It is recognized that the theory of antithetic alternation, as elaborated by Professor Bower, affords a consistent and satisfactory explanation, if the assumptions necessitated by the theory are granted. The present theory, which is put forward merely as a provisional hypothesis, is founded on another class of facts.

With the spread of algal organisms to the land, where in the absence of any vegetation affording shade some at least would be exposed to more intense illumination, the flattened form would probably be assumed. Prolonged drought and the influence of direct sunlight, inducing directly a change of form into a cylindrical body, might be accompanied by the substitution of a reproductive organ forming dry reproductive cells (spores) for those adapted to an aquatic existence. The acquisition of more highly developed absorbent organs (primitive roots) would further the existence and

growth of this modified gametophyte. This spore-producing stage would at first follow the sexual stage in any individual exposed to dry conditions. It is possible to imagine, however, how the association of the asexual with the sexual individual might come about. Absence of fluid water would prevent the liberation of motile spores from the zygote. The latter would be obliged to germinate *in situ*, and the fact that it did so under dry conditions would tend to the shortening of the sexual stage, and the speedy assumption of the sporophytic form and mode of reproduction. From the spore, which would always separate from the parent, a sexual individual would arise, since germination could only take place in a damp spot. As soon as, with the increase in size and complexity of the spore-bearing plant, a vegetation capable of affording shade came into existence, the conditions suitable for the persistence of the more primitive, alga-like, sexual stage in the life-history would be present. The latter has, of course, also been modified in various ways.

In the concluding portion of this paper, the theories of antithetic and homologous alternation are compared by considering the explanations they afford of the facts. The general conclusion reached is that, while both afford a *possible* explanation of the facts of alternation in archegoniate plants, any evidence which would render one or the other untenable is wanting. The reasons on which either is considered more probable depend on the views held as to the lines of descent which have been followed, and the degree to which the different groups of archegoniate plants have had a common origin, or represent actual steps in the process of evolution of the sporophyte. Under these circumstances the question must be regarded as an open one until the available lines of evidence have been more fully investigated.

I am especially indebted to Dr. Scott and Professor Bower for their assistance and advice; the work was commenced in the Jodrell Laboratory of the Royal Gardens, Kew, and subsequently carried on in the Botanical Laboratory of the University of Glasgow.

THE LIGULE IN LEPIDOSTROBUS.—The presence of a ligule on the vegetative leaves of *Lepidodendron* is now a well-ascertained fact, and it can be readily seen in several of the slides preserved in the Williamson Collection at the Natural History Museum. The best description and figures of the ligule in the

vegetative region are those given by M. Hovelacque in his admirable memoir on *Lepidodendron selaginoides*[1], in which species the ligule is shown on the upper surface of the leaf-cushion, enclosed in a deep ligular pit penetrating far into the tissue of the leaf-base. This ligular chamber opens to the exterior only by a small pore situated just above the upper angle of the scar from which the leaf-blade separated; the small pore probably corresponds with the mark first described by Stur[2] from the casts, and considered by him to be the ligular pit. The ligule itself is described by Hovelacque[3] as having the form of a triangular pyramid with blunt angles and point; its insertion at the bottom of the chamber is very oblique, while it is so short as to be generally invisible from the exterior.

Hitherto, however, the ligule in the strobilus (*Lepidostrobus*) has not been described, the only reference which I have been able to find in the literature being a not very clear statement by Solms-Laubach, who writes, referring to some impressions found in the Gegenort mine at Dutweiler: 'I observe on several of the leaves, close to where the line of fracture passes through their bases, a small obtusely triangular scar with a trace-point in its centre, which from its median position is probably the object discovered by Stur on the barren cushion, and called by him the ligular pit. Its occurrence in Lepidostrobi was till now unknown[4].'

Recently, in an examination of the Williamson Lepidostrobi undertaken under the direction of Dr. Scott, F.R.S., I have found the ligule sufficiently well preserved to enable one to make out not only its position but also some details of its structure. It can readily be seen in several of the Williamson slides (C. N. 568, 574, 1776 A, 1776 B, 1776 C), and I have also seen it in a slide (S. 615) kindly lent by Dr. Scott.

In the accompanying figure (from C. N. 1776 C), the expanded, peltate, distal extremity of the horizontal portion of the sporophyll is shown at *A*, with part of the vascular bundle at *B*, and many of the short tracheides (barred cells) so characteristic of this region of

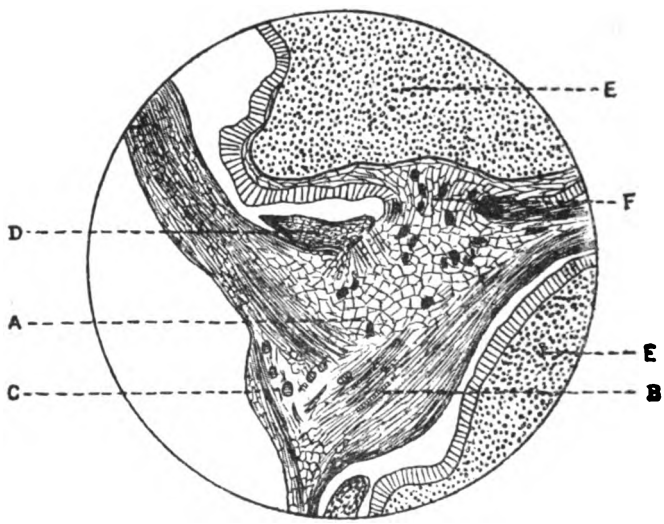
[1] Recherches sur le *Lepidodendron selaginoides*, Stern: Mémoires de la Société Linnéenne de Normandie, Caen, 1892.

[2] Die Culmflora der Ostrauer und Waldenburger Schichten: Abh. d. k. k. Geol. Reichsanstalt zu Wien, Vol. viii, Heft ii (1877), Taf. xix, Fig. i. l.

[3] Loc. cit., p. 155.

[4] Fossil Botany, English edition, pp. 234, 235.

the sporophyll at *C*. The ligule *D* is shown arising freely from the upper surface of the sporophyll just where the latter bends upwards to form one of the covering scales of the cone. At *E* the distal ends of two sporangia are shown, each filled with spores; while at *F* is seen a 'pad' of tissue forming a portion of the attachment of the upper sporangium to the sporophyll, the dark bodies having probably been 'mucilage-cells.'



WOODCUT 1.—Nearly radial section of the periphery of the cone of *Lepidostrobus* (×32: semi-diagrammatic). *A* peltate expanded portion of sporophyll, with *B* its vascular bundle; *C* short tracheides; *D* the ligule; *E* sporangia and spores; *F* pad supporting sporangium.

In another slide (C. N. 568) the ligule is seen in transverse section, showing a triangular form with one angle directed inwards, and very similar to sections figured in Hovelacque [1].

The length of the ligule shown in the figure is about ·5 mm., and the tissue consists mainly of a very small-celled parenchyma, the cells of which exhibit dense contents, making it probable that they were of the nature of mucilage-cells. Near the base is to be seen a transverse row of four or five larger clear cells possibly representing the glosso-

[1] Loc. cit.; cp. Fig. on p. 95, and T. xvii, Pl. vii, Fig. 2.

podium described by Harvey Gibson in *Selaginella*[1]. These cells are well differentiated from the other cells of the ligule by their relatively large size and absence of contents, and between these clear cells and the ordinary tissue of the sporophyll are some large dense-looking cells, showing what appear to be conspicuous nuclei, which may be compared with the similarly situated dense cells shown by Harvey Gibson in *Selaginella spinosa*[2].

The base of the ligule is at a considerable distance from the leaf-trace bundle, and I can see no trace of tracheides or barred cells either in the ligule itself or in the tissue intervening between it and the leaf-trace bundle. There is no evidence of any special arrangement of the vascular tissues in relation to the ligule such as is described by Harvey Gibson in *Selaginella helvetica* and other forms, but there is seen a distinct convergence of the cell-rows towards the point of insertion as shown in the drawing.

To sum up then, the position of the ligule in *Lepidostrobus*, with the sporangium between it and the axis, is identical with that in *Selaginella*; but, whereas in the latter genus it is quite close to the axis of the cone, in the former the great elongation of the sporangium which had taken place in the radial direction had of course carried the ligule with it, and so the latter comes to be situated near the periphery of the cone and at a considerable distance (1.5 centim.) from the axis. The whole of the horizontal (sporangium-bearing) portion of the sporophyll thus appears to be homologous with the short leaf-base or cushion on the vegetative stem. The ligule differs markedly from those exhibited on the vegetative leaves of *L. selaginoides*, &c., in the complete absence of a ligular chamber; although, as can be seen in the drawing, it is overarched by the protuberant distal end of the sporangium.

In conclusion, I have to express my indebtedness to Dr. Scott, F.R.S., for kind help.

ARTHUR J. MASLEN.

ROYAL COLLEGE OF SCIENCE, S.W.

[1] Contributions towards a Knowledge of the Anatomy of the Genus *Selaginella*, Spr.; Part II, The Ligule: Annals of Botany, Vol. x.

[2] Loc. cit., Figs. 3 and 6.

The Fertilization of Onoclea[1].

BY

WALTER R. SHAW.

With Plate XIX.

THE details of the process by which the sexual cells and their nuclei unite have not been exhaustively studied in very many plants. Most of the contributions to the subject are the results of investigations into the development during extended periods of the plants under observation, so that necessarily the particular subject of cell-conjugation and nuclear fusion has received proportionally less time and attention. This is more especially true with regard to the Characeae, the Bryophytes, and the Pteridophytes.

LITERATURE.

The accounts of fertilization in plants all indicate that the process consists in the fusion of two nuclei in the resting condition. This has been described by Wager[2] for *Cystopus*, by Harper[3] for *Sphaerotheca* and *Erysiphe*, by Oltmanns[4] for *Vaucheria*, by Klebahn[5] for *Oedogonium*, and by Farmer and

[1] Prepared under the direction of Professor Douglas H. Campbell in the Botanical Laboratory of Leland Stanford Junior University.

[2] Wager '96. p. 331.

[3] Harper '96, pp. 656 and 659.

[4] Oltmanns '95, p. 401.

[5] Klebahn '92, p. 252.

[Annals of Botany, Vol. XII. No. XLVII. September, 1898.]

Williams[1] and by Strasburger[2] for *Fucus*. The approaching sexual nuclei and the resulting spore-nucleus have been described by Klebahn[3] for *Closterium* and *Cosmarium* and for *Rhopalodia*[4], and by Fairchild[5] for *Basidiobolus*. In *Vaucheria* the sperm-nucleus becomes nearly like the egg-nucleus before the two come in contact. In *Oedogonium* the chromatin granules of the sperm-nucleus are larger than those of the egg-nucleus at the time when fusion begins. In *Fucus* the sperm-nucleus becomes closely appressed to the egg-nucleus before the granular structure of the sperm-chromatin appears. From Strasburger's[6] work on the Gymnosperms it is evident that the sexual nuclei in these plants are alike and nearly equal at the time of fusion, and in this respect the sexual nuclei of *Lilium Martagon* are similar, according to the account of Guignard[7]. Mottier[8] has recently found that, contrary to the description by Guignard of *L. Martagon*, the constituents of the male and female nuclei of *Lilium candidum* cannot be distinguished in the nucleus formed by the fusion.

The general character of the sexual organs and the sexual cells of the Ferns is too well known to need reviewing here. The way in which the sexual organs open and the multiciliate spermatozoids make their way from the antheridium to the archegonium is described in many text-books[9]. It is with the development and structure of the spermatozoid with reference to its constituents and their distribution and activities that investigation has lately been mainly concerned. Guignard[10] had in 1889 concluded that the body of the spermatozoid is formed from cytoplasmic as well as nuclear substance: and, according to Strasburger[11], Belajeff had in the same year advanced so far as to describe the spermatozoid

[1] Farmer and Williams '96, p. 482.
[2] Strasburger '97, p. 351.
[3] Klebahn '91, p. 440.
[4] Klebahn '96, p. 639.
[5] Fairchild '97, p. 292.
[6] Strasburger '78, pp. 50–51; see also Vines '95, p. 472.
[7] Guignard '91, p. 198; see also Wilson '96, p. 161.
[8] Mottier '97, p. 149.
[9] See Strasburger and Hillhouse '89, pp. 292–295; also Atkinson '94, p. 20.
[10] Guignard '89, p. 379.
[11] Strasburger '92, p. 105.

as consisting of a chromatic body formed from the nucleus of the mother-cell, and an achromatic band formed from the cytoplasm of the mother-cell. Strasburger himself described in detail the development of the spermatozoid of *Osmunda*[1], and the mature spermatozoid of *Phegopteris Giesbrechtii*[2] as it appeared when stained with fuchsin-iodine green. He found the two anterior coils to stain red, and these he considered to be of cytoplasmic origin; while the posterior coil took the blue colour characteristic of the nucleus. The cilia he found distributed along the forward coil some distance from the end, and the middle coil, he suggested, might have contained the centrosome, if there had been one. At present[3] he is inclined to think that there is no individualized centrosome in the spermatozoid of Pteridophytes. He found by treating the spermatozoid of *Marsilia*[4] with the same stain and allowing it to fade, that only the hinder and larger of the ten or twelve coils contain the nucleus, which extends forward to the region where the cilia are attached. In following the development of the spermatozoid of *Onoclea*, Campbell[5] was unable to find that so large a part of the forward end is of cytoplasmic origin as Strasburger[6] described for *Osmunda* and *Phegopteris*. 'The body of the free spermatozoid,' the former writes, 'has the form of a flattened band with thickened edges, which tapers to a fine point at the anterior end, but is broader and blunter behind[7].' This description fits the living spermatozoid as it is to be seen when caught in the slime about the open archegonia.

The principal accounts of the spermatozoid after its entrance into the egg are those of Campbell. In studying the development of *Pilularia*[8] he found the spermatozoid within the egg close to the egg-nucleus. It had become a spherical granular nucleus darker than the egg-nucleus and with little more than

[1] Strasburger '92, p. 114.
[2] l. c., p. 116.
[3] Id. '97, p. 420.
[4] Id. '92, p. 122.
[5] Campbell '95, p. 312.
[6] Strasburger '92, pp. 115-116.
[7] Campbell '95, p. 313.
[8] Id. '88, p. 249; '95, p. 405; for fig. cf. also Wilson '96, p. 106.

half the diameter of the latter. In following the development of *Osmunda*[1] he found that the spermatozoid loses its pyramidally coiled form after it comes in contact with the egg-nucleus and before the two fuse. In his work with *Marattia*[2] he found the spirally coiled spermatozoid in contact with the egg-nucleus. In a later stage he found two nuclei present which seemed to be the male and female nuclei. They were not very different in size or appearance. He concluded that the female nucleus had become smaller, and that the spermatozoid had changed into a similar nucleus. 'The two nuclei,' he writes, 'then gradually fuse, but all the different stages could not be traced. Before the first division takes place, however, but one nucleus can be seen, and this nucleus resembles the nucleus of the unfertilized egg.' From these accounts it has been concluded that the spermatozoid of the Ferns enters the egg in the condition in which it swims free, and then, on coming near to or in contact with the egg-nucleus, it undergoes a change in structure which makes it more like the latter and also like the resting nucleus in the sperm-cell of the antheridium. In this respect the details of fertilization are much alike in many plants, and the behaviour of the animal spermatozoön is strikingly similar. This is shown by Van Beneden's account of fertilization of *Ascaris*[3], in which the spermatozoön, after entering the egg, rapidly changes and forms a typical nucleus exactly similar to the egg-nucleus.

Preliminary Investigation.

The differences in size, structure, and habits between the male and the female gametes are greatest in those plants in which the condition of the gametes during fertilization has been least studied: viz. the Archegoniatae and the Characeae. In the fall of 1895 the writer made some microtome-sections

[1] Campbell '92, p. 70; '95, p. 348. [2] Id. '94, p. 9; '95, p. 261.
[3] Wilson '96, p. 133 (refers to Van Beneden 1883–87–88).

of the oogonium of *Chara* with a view to finding the spermatozoid on its way to the egg-nucleus. In the species which were studied, no sign could be found to indicate at what stage the spermatozoid entered the oogonium, and no spermatozoids were found in the sections. At that time Dr. D. H. Campbell suggested that some of the Ferns would be better subjects for following the stages which were sought, and he kindly supplied spores of *Marsilia vestita* and also another species of *Marsilia* (*M. Drummondii*?) from Australia and *Onoclea sensibilis*.

When the spores of *Marsilia* are sown in water, the prothallia become fully developed in about fifteen hours[1] at ordinary temperatures; at the end of that time the spermatozoids are set free, and make their way to the female prothallium and through the large mucilaginous funnel to the archegonium, where they sometimes arrive before that organ is open. The first division of the egg takes place within about an hour[2] after the entrance of the spermatozoid into the archegonium. It was found difficult to mark the exact time at which the spermatozoid enters. While endeavouring to become sufficiently familiar with the plant to overcome this difficulty the writer found that in some cases the oospores of the Australian *Marsilia* developed into embryos without any evidence of the presence of spermatozoids about the archegonia. The spores had been sown about ten o'clock at night, and were first examined about half-past eight the next morning. No spermatozoids were seen during that morning, and there were no remains of spermatozoids about the mucilaginous funnel in front of the archegonium. The prothallia were kept, however, and after a few days they were found to bear embryos of considerable size. This suggested the idea that the embryos might have been developed without fertilization, and a short series of experiments[3] was made with isolated macrospores for the purpose of testing the matter, and indicated that such was

[1] Campbell '95, p. 405.

[2] l. c., p. 407.

[3] Shaw '97, p. 114.

the case. In the light of this result the writer's experience with *Chara* affords ground for questioning whether parthenogenesis in that genus is confined to the dioecious species, *C. crinita.*

The spores of *Onoclea sensibilis* had been brought by Dr. Campbell from Massachusetts. They were sown in the laboratory, some in September, 1895, and others in January, 1896. When the earlier culture was examined in January, some of the female prothallia were found to have formed mature archegonia, but when they were placed in water most of the archegonia were slow to open. Spermatozoids were set free in a few minutes after male prothallia were placed in water. On February 26 spermatozoids crowded into and about the open archegonia within eight minutes after the prothallia were placed in distilled water. In *Marsilia* the first division of the embryo takes place within an hour after fertilization, and in *Pilularia* after about three hours[1]. Dr. Campbell, by his extensive studies of Fern embryology, was led to believe that the first division of the egg of *Onoclea* occurred about twenty-four hours after fertilization. It was desired to obtain a series of prothallia which would represent as completely as possible the period intervening between the entrance of the spermatozoid into the archegonium and the first division of the egg, for it was the intention at the outset to describe the nuclear and cytoplasmic structures and changes within the egg during that period. On February 26 and the two following days, prothallia were left on the surface of distilled water for a variety of periods of from about ten minutes to fourteen hours and then fixed. On March 4 a similar series was made, which extended the period to twenty-four hours. From the cultures sown in the middle of January, prothallia were used on March 23 to extend the period to thirty hours, and on April 23 to seventy-two hours. Prothallia from each series were sectioned, but in none of them were any embryos or signs of

[1] Campbell '95, p. 407.

division of the egg found. Finally, a double series was begun on May 11 by flooding some of the prothallia in the saucers in which they had grown, and by transferring others from the soil to the surface of distilled water, as had been done with the specimens of all the previous series. Those left on the soil were flooded for a time sufficient for the impregnation of all the mature eggs and then drained. Specimens from the soil were fixed at the end of every twenty-four hours for fifteen days, and from the water at the same times for the first seven days. It was thought, naturally enough, that seven days would be more than enough for the development of embryos, and it was intended that this double series should show whether the growth of the embryos was prevented or retarded by removing the prothallia from the surface of the soil and leaving them on the surface of distilled water. The material was preserved in alcohol, and when sections were made, in the following September, no embryos were found in the prothallia fixed at the end of seven days, and the writer made the mistake of not sectioning a large number of those from the fifteen-day period.

Dr. Campbell then obtained from Michigan spores of *O. Struthiopteris*, and several cultures were started, some in the saucers used in the preceding season, some in a box on soil already in the laboratory, and some in a similar box on a fresh supply of black soil, a mixture of leaf mould and adobe. The plants in the saucers were not well lighted, and for this and other reasons they did not grow so rapidly or uniformly as those in the boxes. Each box was covered with a pane of glass. The spores on the old soil were sown on November 3, and the box was inclined at an angle of 30° before a deep south window about 60 cm. distant. Those on the fresh soil were sown on November 17, and placed in the same way before an east window. The tilted position of the boxes secured a better illumination, and kept the surface of the soil well drained. It also prevented the water which condensed on the inside of the glass cover at night from dripping on the mature prothallia and thus fertilizing

the ripe archegonia. Many of the plants of the culture of November 3 were ready for fertilization in less than two months. The soil of this culture was poorly mixed, so that the plants on different parts of its surface did not develop uniformly, but they were quite capable of producing embryos.

The first series (No. I) of *O. Struthiopteris* was begun December 29, 1896. Three lots were fertilized in as many ways: lot 1 included prothallia which were removed from the soil, fertilized under the microscope, and replaced on moist soil in watch-glasses; in lot 2 were plants which were removed from the box with soil attached to their root-hairs, and fertilized by flooding them in watch-glasses, where they were drained and left; lot 3 was fertilized by flooding the prothallia on the soil. Others were treated like the first lot, except that they were not passed under the microscope. Ten specimens from lot 2 were placed in water under the microscope eight days afterwards, and archegonia opened on only two of them. This indicated that the formation of mature archegonia had ceased on most of the prothallia of this lot, probably because the fertilization was effective. Plants from different lots were fixed on each successive day for ten days. Specimens representing the whole series were sectioned, but no embryos were found.

On February 15, 1897, the culture of November 17, 1896, was well advanced. The prothallia were the greenest and most regularly formed of all that had been raised. The box was placed in a horizontal position, and filled for about fifteen minutes with water enough to flood half of the culture. The box was then drained and returned to its original position so as to minimize the geotropic disturbance. A series (No. III) was then taken from the box after varying intervals up to nine days and twenty-three and a half hours. When the oldest of these were sectioned, one egg with the nucleus in the anaphase of the first division, and another with the first division complete, were found. In the next younger lot of this series, which was six days and nineteen hours old, no dividing egg or embryo was found. With this clue to

the fact that the first division of the egg takes place nine or ten days after fertilization, more of the older stages from Series I were sectioned and the following young embryos were found:—

Embryos of *Onoclea Struthiopteris* from Series I.

Fertilized 9 days, 3 embryos of 2 cells.

„ 10 „ { 2 „ 2 „ ; 2 „ 4 „ ; 1 „ 8 „

On March 18 both box cultures were flooded (one had been half-flooded before), and from each specimens were fixed daily to form a series, one covering fifteen and the other twenty days. With thermometers, which hung near the cultures, the temperature of the room was noted each day when plants were fixed, and recorded with the maximum and minimum temperatures for the preceding twenty-four hours. It ranged from 10° C. to 25° C. The material yielded the following:—

Embryos of *Onoclea Struthiopteris* from Series IV and V.

Fertilized 10 days { 2 embryos of 4 cells. ; 1 „ 6 „ ; 1 „ 15 „ ; 2 „ 16 „ ; 1 „ 17 „

„ 12 „ 1 „ 16 „

The results were enough to satisfy the writer that, under favourable conditions in the laboratory, the egg of *Onoclea Struthiopteris* is not likely to divide sooner than a week after fertilization; and they indicated that uprooting, handling, and replanting the prothallia did not retard the formation of the embryo.

After the embryos of *O. Struthiopteris* had been found, attention was again turned to the longest series of prothallia which had been killed in 1896, and by sectioning a large number of prothallia the following were obtained:—

Embryos of *Onoclea sensibilis* from Series G.

Fertilized 8 days, 1 embryo of 2 cells.

„	10	„	2	„	2 „
„	12	„	2	„	4 „
			3	„	8 „
			3	„	16 „
„	15	„	1	„	32 „
			1	„	130 μ long.

This showed clearly that we should not expect to find the first division of the egg in these prothallia less than a week after fertilization, and, conversely, that the absence of embryos in the shorter series which had been prepared does not by itself indicate that they were abnormal. It was unfortunate that the specimens kept on the surface of the water did not cover a period longer than seven days. This failure was repaired as far as possible by fixing such a series from the cultures of the second species studied, but these plants were killed too late to be available for present purposes. It is also regretted that the long series of *O. sensibilis* was obtained after the prothallia had grown to a large size, and bore such large numbers of archegonia as to make the study of fertilization in these plants a more complex problem; for at some certain time after reaching maturity the egg, if unfertilized, becomes atrophied to such an extent as to render normal fertilization impossible. Yet spermatozoids enter such eggs, and it has been suggested that their presence may then have some retarding influence on the development of the normally fertilized eggs on the same prothallium. On account of the long period occupied by the stages which it was proposed to follow, these Ferns are less suitable for the research than was expected.

METHODS.

The cultures of *Onoclea sensibilis* were obtained by sowing spores on sterilized soil in earthenware saucers. Cultures

started in September were ready for fertilization-experiments in the following February, and those started in January were sufficiently mature for the same purpose two months after the spores were sown. A series of trials showed that when female prothallia bearing mature archegonia were placed in water, the archegonia opened within five minutes, and when male prothallia were present the open archegonia were all entered by spermatozoids within three minutes. It was found convenient to carefully transfer large numbers of prothallia from the soil to the surface of water in watch-glasses. A few minutes or an hour later, the prothallia were examined, and those with no open archegonia were rejected. Then, after the lapse of the desired periods, specimens were taken from the watch-glasses and placed in the fixing agent. All of the series used, except Series G, were fertilized in the manner described. The plants of the latter series, as already explained (p. 267), were fertilized on the soil and not removed until the time for fixing. Unless otherwise specified the observations refer to *Onoclea sensibilis*. In the following table of the series of prothallia killed, the italics show the material used:—

Sowing of culture.	Series.	Beginning of Series.	Periods between fertilization and fixation.	Chromic acid fixing solution.	Hours in fixing agent.
Sept. '95	B	Feb. 26, '96	*10, 20, 30* minutes, *1, 2, 3, 4, 5,* 6, *7,* 8, 10, 12, *14* hours.	1%	18–24
Sept. '95	C	March 4, '96	6, 8, 10, 12, 14, *24* hours.	1%	24
Sept. '95	D	March 9, '96	1, 2, 3, 4, 5, 6, 7, 8, 9, *10* hours.	1%	6–8
Jan. 10–17, '96	E	March 23, '96	*1,* 2, 3, *4, 5,* 6, 7, *8,* 9, *10, 12, 14, 16, 18, 20,* 22, 24, 26, 28, *30* hours.	½%	8–24
Jan. 10–17, '96	F	April 23, '96	1, 2, 3, 4, 5, *6,* 8, 10, 12, 14, 16, 18, *20,* 22, 24, 28, 32, *36,* 40, *44, 48, 60, 72* hours.	1%	24
Jan. 10–17, '96	G	May 11, '96	1, 2, 3, 4, 5, 6, 7, 8, 9, *10,* 11, *12,* 13, 14, *15* days.	1%	24

For fixing[1] the prothallia they were placed whole in ½% or 1% chromic acid. The best results were obtained by leaving the specimens in ½% chromic acid for eight to twenty-four hours. In most cases dilute sulphurous acid was used for removing the last traces of chromic acid.

Most of the material was stained with Czokor's alum-cochineal, in which the prothallia were allowed to remain for eighteen to twenty-four hours. Some of the plants were stained in alum-carmine, and others in diluted Delafield's haematoxylin. Some of the sections of plants which were stained *in toto* in alum-cochineal were counter-stained on the slide with Bismark brown. A few sections were restained on the slide with Flemming's triple stain[2], safranin-gentian violet-orange G, and a few others with Heidenhain's iron-haematoxylin.

The prothallia were sectioned in paraffin. At first turpentine was used as a medium between alcohol and paraffin, but it was soon discarded, and xylene used according to the method recommended by Zimmermann[3] in his handbook. The wings were cut off with a razor before the prothallia were imbedded. The sections were cut 10 μ thick on a Minot microtome of the older style. They were cleared with clove oil and mounted in Canada balsam.

INVESTIGATION.

The spermatozoids, which were usually held in large numbers in the slime before the mouth of the archegonium, remained unchanged for a long time, and were favourable objects for study. They were in a position to be acted upon very quickly by the fixing agent, and also by the stain. Those fixed after fourteen, eighteen, and twenty-four hours had the same appearance as those fixed after a few minutes. They had all lost the nutritive vesicle, and become a little

[1] For fixing the series of *O. Struthiopteris* most of the agents given in Zimmermann's Botanical Microtechnique were used.

[2] Zimmermann '93, p. 186.

[3] l. c., pp. 32-33.

drawn out on entering the slime. With all the stains used the darkest part of the body is a corkscrew-shaped homogeneous rod, oval in cross-section, which tapers gradually forward and abruptly backward. It does not terminate in a sharp point at either end. In all the specimens which were noted closely the spiral turns of the body are in the direction of a left-hand screw. Webber[1] finds that the helicoid ciliated band in the spermatozoid of *Zamia* is coiled in the same direction. The number of turns varies with the length to which the corkscrew is extended: when shortened, like a watch-spring, it makes two turns (Fig. 10 *b*); just outside the archegonium it usually makes about three and a half turns (Fig. 5); in the canal, when it becomes most extended, it may make five turns (Fig. 1). In all the forms an almost stainless band can be seen attached to the forward edge of the corkscrew, and extending beyond its forward point. This band is broadest toward the forward end, and it tapers backward to the thickest part of the corkscrew rod. The outer edge of the band appears to be thicker than the rest, and the thickening is greatest near the forward end. The dark rod is assumed to be the nucleus, and the wing-like band the cytoplasmic portion of the spermatozoid. The cilia did not usually stain enough to be visible; but when Heidenhain's iron-haematoxylin was left rather dark they could be seen distinctly enough to show that some extended forward and others backward. They could not be traced to their respective points of attachment, and so they were omitted from the drawing (Fig. 8) which was made from such a specimen. No centrosome was seen.

The nucleus of the spermatozoid has the same form as that figured by Campbell[2] for the developing spermatozoid of *O. Struthiopteris*. It extends through the greater part of the length of the body. In this respect it differs from the nucleus which Strasburger[3] found in the spermatozoid of *Phegopteris*. In the latter it occupied only the larger pos-

[1] Webber '97, p. 17. [2] Campbell '95, p. 131.
[3] Strasburger '92, p. 116.

terior turn of the corkscrew. The spermatozoid of *O. sensibilis* is like that of *Chara*, as described by Belajeff[1], in having the cytoplasm extending along the nucleus. The thickened outer edge of the cytoplasmic band may correspond to the 'Rückfäden' which Belajeff was able to distinguish in the forward cytoplasm of the spermatozoid of *Chara* until a late stage in its development.

Long before the archegonium opens the egg-nucleus comes to the resting condition, and contains one or more nucleoli. The ventral canal-cell is the smallest cell in the archegonium. After it is formed, the pressure of the egg makes it still smaller and concave, and its nucleus becomes flattened. In later stages the walls of the ventral canal-cell swell up, and by pressure cause the egg to become concave on the outer side, which later forms the receptive spot. In these stages the egg-nucleus also is flattened and concave. The egg is in this condition when the archegonium opens. In the living sections, under the microscope, the writer observed the egg to swell as soon as the canal was cleared of its dissolving contents, and fill up the venter. If many spermatozoids were near, they swarmed into the canal, and a large number made their way into the venter, where they swarmed about freely, quite differently from those in the close quarters of the neck, which were motionless or moved slowly. Often spermatozoids which had entered the venter found the canal again, and made their way out, slowly through the narrower portion of the canal, but rapidly in the wider part. On first entering the slime discharged by the archegonium, the spermatozoids left their trophoplasmic vesicles behind, and their motion was retarded. It was the resistance of the slime which pulled off the vesicles. In the canal the slimy mucilage seemed to be denser, and when a spermatozoid entered it, the corkscrew spiral became drawn out, and the number of turns increased, and the forward motion of the spermatozoid was accompanied by a rotation which corresponded to the pitch of the screw.

[1] Belajeff '94, p. 43.

All the sections of prothallia that were killed within an hour after the entrance of the spermatozoids into the archegonia, show the eggs in a collapsed condition (Figs. 1 and 2), concave on the outer side, and the nucleus in each conforms to the shape of the cytoplasm. In this state the shape of the egg is about the same as it is in the unopened archegonium after the canal-cells have swelled. The return to this form may have been due to the action of the fixing or imbedding agents, the egg in this stage being more susceptible to their shrinking influence, either because it is not at this time in the state of tension which it acquires later, or because the open canal permits the rapid access of the plasmolizing agents. There are reasons to believe, however, that the collapse is not an artificial plasmolysis, but that it takes place as soon as the spermatozoid enters the egg. The mature egg has been described (for the other species) as having a large hyaline receptive spot[1]. The concavity of the collapsed egg occupies the position of that spot. That it was formed before the plants were killed seems evident from the movement of a number of spermatozoids in the venter. This can be seen in the living plants. That the number of these spermatozoids is large is shown by the specimens stained and sectioned. They can hardly have been carried into the venter by the fixing agent, for those in the canal were fixed first, in the extended condition, and those in the venter afterward, in the contracted form. From the evidence at hand it appears that as soon as the egg is entered by a spermatozoid it loses its turgidity, and the spermatozoids which come into the venter afterward meet with little or no resistance from the egg. It may be that the turgid condition of the egg, in the first place, offers mechanical facility for the screw-like spermatozoid coming through the narrow base of the neck to force itself into the cytoplasm of the receptive spot; and that the plasmolytic condition of the egg afterward deprives the following spermatozoids of this advantage, and protects

[1] Campbell '95, Fig. 159.

the egg from injury or from multiple fertilization by them. A few careful experiments in fixing archegonia before and after the first spermatozoid enters the egg ought to reveal the truth of the matter.

Within half an hour after the entrance of the spermatozoids into the archegonium the canal is practically closed by the expansion of the four proximal neck-cells and the four just beyond them. The egg gradually recovers its turgidity and forces the free spermatozoids against the outer wall of the venter (Fig. 3). The membrane which, from analogy with the development of the eggs in other plants, we would expect to form around the egg immediately after the entrance of the spermatozoids, was not seen in any of the earlier stages. If the membrane is of the nature of cellulose it ought to be brought out distinctly by the Bismark-brown with which the subject of Fig. 1 was stained on the slide. This stain colours even the cytoplasm in this case. In all the preparations which were examined there was no evidence that a membrane of appreciable thickness is formed immediately after the entrance of the spermatozoid or for some time afterward. A conclusion so contrary to analogy must remain in doubt. It may be that the chromic acid used for too long a time destroyed the membrane.

The early history of the spermatozoid inside the egg was not satisfactorily followed. After the ten- and twenty-minute periods the collapsed state of the egg interfered with the study of the enclosed spermatozoid, and the stains used for these stages were not the best. The difficulty was increased by the free spermatozoids crowding into the concavity of the egg. So the mode of entrance of the spermatozoid into the egg cannot now be described. In one case the spermatozoid appeared to be still outside the egg-nucleus, against which it lay in an open coil after an hour, but in other cases it was found inside the egg-nucleus within thirty minutes. The nucleus of the spermatozoid undergoes no visible change in structure while in the egg-cytoplasm. Whether the cytoplasmic wing and the cilia of the spermatozoid are taken

into the egg-nucleus could not be shown with the faint cytoplasmic stains used at these stages. We may reasonably expect to find that they are left outside in the egg-cytoplasm, but it is a question which will require to be settled by actual observation. In Figs. 3 and 11, which were drawn before the structure of the free spermatozoid was understood, there is something in the egg-cytoplasm on the outer side of the egg-nucleus which strongly suggests by its general appearance that it is the remains of the sperm-cytoplasm, accompanied in the case of Fig. 3 by the cilia. The writer has lately observed, in a specimen stained with Heidenhain's haematoxylin, something that looks like a loose bunch of cilia in the same position; and in some horizontal sections the outer part of the egg-cytoplasm, when seen from the outer side, shows radiations which have a spiral twist, such as the cilia of the spermatozoid sometimes show when that body is viewed from in front [1].

The egg-nucleus at the beginning of fertilization, and all through the process, is in the typical resting condition. The nucleoli, for there are generally several, are the most conspicuous structures in the stained sections. They vary in size and present a peculiar porous structure. The linin appears as a delicate network which bears the very small chromatin bodies. This was not demonstrated for all stages, but the general appearance of the female nucleus is the same throughout the process, and in later stages some well-stained examples made it possible to observe the linin network and chromatin bodies minutely (Fig. 12). Nothing was seen to indicate that the whole nuclear 'membrane,' if it may be called such, was dissolved. There were indications that it dissolves or is ruptured at the place where the sperm-nucleus enters. The most remarkable fact observed, and one about which there is no doubt, is that the sperm-nucleus enters the egg-nucleus before it changes in form or visible structure. This is clearly shown in the section represented

[1] Webber has shown ('97, 2, p. 227) that in the fertilization of *Zamia* the cilia are left behind soon after the entrance of the spermatozoid into the egg-cell.

in Fig. 1 and sections of the other eggs on the same slide. In Fig. 1 the small end of the spermatozoid is directed toward the base of the archegonium. The lower portions of two coils were cut off by the razor, and are to be seen in the next section on the slide. The sperm-nucleus within the egg-nucleus becomes granular and the granules slowly separate. Thirty minutes after the entrance of the first spermatozoid into the archegonium the sperm-nucleus may show traces of the granular structure, as in Fig. 2, but usually it is not evident until two hours have elapsed. It shows plainly in Fig. 3, from a specimen fixed after three hours. Fig. 4, after twenty-four hours, shows little, if any, advance in this respect. Fig. 6, after twenty-four hours, shows the sperm-chromatin distributed in one quarter of the egg-nucleus very much as it was found in Fig. 9 after sixty hours. In most of the preparations the chromatin of the two nuclei can be seen distinctly, but in only a few cases was the linin also clearly distinguished. Among the best of these is one represented in Fig. 12, which was fixed in ½ % chromic acid for twelve hours, washed two days in water, one and a half days in dilute sulphurous acid, and three hours in water, and then stained with alum-carmine. The linin threads connecting and supporting the chromatin granules of the female nucleus are especially distinct, and had one the time one might almost construct a complete map of the network system. In the sperm-nucleus the granules and the threads are so closely packed that the courses of the threads cannot be followed in detail. The sperm-nucleus often retains the spiral arrangement of its substance for a long time, as in Fig. 13, after twelve hours. It may lose this arrangement early, as in Fig. 7, after fourteen hours. Here the larger and smaller end can still be identified. In the latest stages in which the sperm-substance could be recognized it had become distributed through a larger part of the female nucleus (Fig. 9).

The different eggs fertilized on one prothallium at the same time do not have the sperm-nucleus in the same

condition. This makes it impossible to say without further study what is the rate of normal nuclear fusion. Among the few early stages of *O. Struthiopteris* that were sectioned and successfully stained, one after two days showed the male nucleus but little further advanced than that of Fig. 14, which was killed after thirty-six hours. Another egg of that species contained, after three days, a nucleus which had slightly enlarged but contained nothing that could be identified as male chromatin, although there were outside of this egg the crowded remains of free spermatozoids which must have entered the venter when the canal was open and the egg in a receptive condition. So it appears that after three days the nuclear fusion may be complete.

The cytoplasm of the egg becomes vacuolated as the cell becomes turgid, and may after a time be pretty evenly distributed around the lumen (Fig. 11); or it may be denser on two sides of the nucleus (Fig. 4). In the material stained with alum-cochineal and Delafield's haematoxylin, the cytoplasm appeared to be composed mainly of spherical bodies. But in the beautiful alum-carmine preparations the cytoplasm, although very slightly stained, showed, with the most favourable illumination, a reticular or alveolar structure resembling that of the nuclear network, but with larger meshes. This was not exhibited distinctly enough to be represented in the drawing. No centrosome or radiations, such as are formed about one, were seen in the egg-cytoplasm.

In most of the eggs that are fertilized the protoplasm decreases in quantity after two or three days, and retreats to the inner side of the venter with the nucleus, which becomes smaller. Whether any such as these afterward divide is not known. A large proportion of the fertilized eggs never divide. It might be supposed that the rather peculiar treatment of the prothallia in these experiments was responsible not only for an increase in the proportion of sterile eggs, but also for a wider variation in the rate of nuclear fusion. Opposed to such a supposition is the fact that many of the prothallia left on water showed more

nuclei in various stages of mitosis than those which were not disturbed until they were fixed.

In an early stage of the segmentation of the egg, an exception was found to the order of the divisions usually described [1]. This was in an embryo in which the epibasal octants had already formed, but the hypobasal quadrants were separated by a median instead of a transverse wall. On one side of this two octants had formed, and on the other the nucleus was in the metaphase of division. In other cases the quadrant walls were more or less oblique.

The absence of radiations in the cytoplasm of the egg during fertilization is a character in which the Fern resembles the lower plants in which the process has been described. Strasburger's suggestion [2] that their absence in *Fucus*, where they occur during mitosis, may be due to the fact that the cell division does not immediately follow fertilization, will be equally applicable to this Fern if the radiations can be found during mitosis. We were led to expect from the accounts of fertilization in other plants that the sperm-nucleus would become more or less like the egg-nucleus before the two united. The entrance of the unchanged sperm-nucleus into the egg-nucleus in *Onoclea* is so notably different from what has been said to occur in the eggs of other Ferns, that a further study of these is very desirable. *Marsilia* and *Pilularia*, while presenting some difficulty with regard to regulating and marking the time at which the spermatozoids enter the archegonia, have the great advantage that the nutrition of the eggs is well provided for, and there are not several eggs to contest for the food from one prothallium. It was thought that the disadvantages of working with the *Onoclea* prothallia could be avoided by selecting young ones on which only one archegonium was ripe, but this was not found practicable when a large number were required. The unsuspected fact that the egg did not divide for more than a week after fertilization was the greatest hindrance to the

[1] Campbell '95, p. 316.

[2] Strasburger '97, p. 419.

completion of this account of fertilization. The quiescent period may be partly or entirely due to imperfect nutrition, and if so it is likely to vary in different closely related species, and in the same species under different conditions. We have found the period to be long in three cultures and two species.

Many of the prothallia used for the present study probably bore, in addition to the eggs fertilized at the recorded times, others which matured later and were then fertilized. This might have been prevented by draining the prothallia, as was done with those which were fixed during the next season. This may be accomplished when the prothallia are removed from the soil to be fertilized, as must be done if they are to be carefully examined, by placing them on moist filter paper in a moist chamber. It will be desirable not only to prevent the fertilization of archegonia which mature later, but also any which have matured earlier than the beginning of the experiment. The most trustworthy account of fertilization will be based on eggs on prothallia on which only one egg is fertilized and that at maturity. Such specimens can be obtained by fertilizing large numbers of prothallia and selecting after ten minutes those on which only one archegonium opens and attracts spermatozoids.

Summary of Results.

1. The body of the free spermatozoid consists of a long corkscrew-shaped nucleus which stains homogeneously, and a lateral band of cytoplasm which extends a short distance in front of the nucleus.

2. The sperm-nucleus enters the egg-nucleus before it changes in form or visible structure.

3. Within the egg-nucleus the chromatin-granules of the sperm-nucleus slowly separate as the meshes of the linin-network slowly enlarge.

4. Throughout the process of fertilization the female nucleus is in the resting condition.

5. The first division of the egg was in no case found until more than a week after fertilization.

6. It is suggested that the egg becomes plasmolysed as soon as the first spermatozoid enters it, and that this serves as a provision against injury by following spermatozoids.

The work which forms the basis of this paper was done in the Botanical Laboratory of Leland Stanford Junior University, with the kind advice and encouragement of Professor Douglas H. Campbell, for which the writer takes pleasure in here expressing his thanks. The paper was written and most of the figures were drawn during the summer of 1897, in the Hopkins Seaside Laboratory, a branch of the University located at Pacific Grove, California.

A detailed account of the development and structure of the spermatozoid of the Ferns was given by Belajeff[1] before the above paper was written, but it had not then been seen by the writer. In that account Belajeff brought out the fact that there is a specially differentiated body in the cytoplasmic band of the spermatozoid which gives rise to the cilia. He described the development of this body (the 'Nebenkern'), from a small body near the nucleus of the 'spermatozoid mother-cell,' into a thread-shaped body in the mature spermatozoid. The writer[2] of the present paper subsequently found these 'Nebenkerne' in the antheridia of *Onoclea* before and during the last cell-division by which the so-called 'spermatozoid mother-cells' are formed. But the cilia-bearing portion of the spermatozoid of the Fern, like that of the Cycad as described by Webber[3], takes no active part in what may be regarded as the essential process of fertilization, and therefore no extended reference to these works need be appended to the foregoing account of fertilization.

[1] Wl. Belajeff, Three preliminary papers in the Berichte d. deut. Bot. Gesell., 1897, p. 337 ff.

[2] W. R. Shaw, Über die Blepharoplasten bei Onoclea und Marsilia, 1898. Soon to appear.

[3] Webber, '97, 2, 227.

LITERATURE CITED.

ATKINSON, G. F., '94: The Biology of Ferns.

BELAJEFF, WL., '94: Ueber Bau und Entwicklung der Spermatozoiden der Pflanzen: Flora, Ergänzungsband, p. 1.

CAMPBELL, D. H., '88: On the Development of *Pilularia globufera*; Annals of Botany, ii, p. 233.

———— '94: Observations on the Development of *Marattia Douglasii*, Baker; Annals of Botany, viii, p. 1.

———— '95: The Structure and Development of the Mosses and Ferns.

FAIRCHILD, D. G., '97: Ueber Kerntheilung und Befruchtung bei *Basidiobolus ranarum*, Eidam; Jahrb. für wiss. Bot., xxx, p. 285.

FARMER AND WILLIAMS, '96: On fertilization and segmentation of the spore in *Fucus*; Annals of Botany, x, p. 479.

GUIGNARD, L., '89: Sur les anthérozoïdes des Marsiliacées et des Équisétacées; Bull. de la Société Bot. de France, xxxvi, p. 378.

———— '91: Nouvelles études sur la fécondation; Annales des Sciences Naturelles, Botanique, Ser. 7, xiv, p. 163.

HARPER, R. A., '96: Ueber das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten; Jahrb. für wiss. Bot., xxix, p. 655.

KLEBAHN, H., '91: Studien über Zygoten: I. Die Keimung von *Closterium* und *Cosmarium*; Jahrb. für wiss. Bot., xxii, p. 415.

———— '92: Studien über Zygoten: II. Die Befruchtung von *Oedogonium Boscii*; Jahrb. für wiss. Bot., xxiv, p. 235.

———— '96: Beitrag zur Kenntniss der Auxosporenbildung: I. *Rhopalodia gibbia* (Ehrenb.) O. Müller; Jahrb. für wiss. Bot., xxix, p. 595.

MOTTIER, D. M., '97: Ueber das Verhalten der Kerne bei der Entwickelung des Embryosacks und die Vorgänge bei der Befruchtung; Jahrb. für wiss. Bot., xxxi, p. 125.

OLTMANNS, F., '95: Ueber die Entwickelung der Sexualorgane bei *Vaucheria*; Flora, 1895, p. 388.

SHAW, W. R., '97: Parthenogenesis in *Marsilia*; Botanical Gazette, xxiv, p. 114.

STRASBURGER, ED., '78: Ueber Befruchtung und Zelltheilung, 1878.

———— '92: Schwärmsporen, Gameten, pflanzliche Spermatozoiden und das Wesen der Befruchtung: Histologische Beiträge, Heft 4, Jena.

———— '97: Kerntheilung und Befruchtung: Jahrb. für wiss. Bot., xxx, pp. 351 and 406.

VINES, S. H., '95: A Student's Text-Book of Botany.

WAGER, H., '96: On the Structure and Reproduction of *Cystopus candidus*: Annals of Botany, x, p. 295.

WEBBER, H. J., '97, 1: The Development of the Antherozoids of *Zamia*; Botanical Gazette, xxiv, p. 16.

———— '97, 2: Notes on the fecundation of *Zamia* and the pollen-tube apparatus of *Gingko*; Bot. Gazette, xxiv, p. 225.

WILSON, E. B., '96: The Cell in Development and Inheritance; London and New York.

ZIMMERMANN, A., '93: Botanical Microtechnique; English translation by Humphrey.

EXPLANATION OF FIGURES IN PLATE XIX.

Illustrating Mr. Shaw's paper on *Onoclea*.

Fig. 1 was drawn with a Leitz objective No. 7, and a Leitz drawing-ocular; all the others were drawn with a Zeiss apochromatic 2·0 mm objective, apert. 1·40; Figs. 5 and 12 with a Zeiss compensation ocular No. 12; the others with a Zeiss compensation ocular No. 8. For sketching the figures (except Fig. 1) the prism section of a Leitz 45° drawing-ocular was screwed to the Zeiss compensation-ocular in place of the upper diaphragm holder.

Magnification determined with a stage micrometer:—Fig. 1 × 500; Fig. 5 × 2,000; all others × 1,200.

All figures are from sections 10 μ thick.

The *arrow* indicates the direction of the growing point of the prothallium.

The *time* given is that which elapsed between the entrance of the first spermatozoids and the fixing of the prothallium.

Onoclea sensibilis.

Fig. 1. One per cent. chromic acid: alum-cochineal and Bismark brown. Vertical section through an open archegonium probably within ten minutes after the entrance of the first spermatozoid. One unchanged spermatozoid is inside the egg-nucleus. Parts of two coils of this spermatozoid were cut off, and appear in the next section of the series.

Fig. 2. One per cent. chromic acid: alum-cochineal. Thirty minutes. Vertical section of the venter of an archegonium containing spermatozoids, and the collapsed egg with a spermatozoid within the nucleus. The canal of this archegonium is almost closed. Two spermatozoids in the section were not drawn.

Fig. 3. One per cent. chromic acid: alum-cochineal. Three hours. Nearly vertical section of the egg. The outside spermatozoids are forced against the venter wall by the expanding egg. Portions of the egg-nucleus, one containing a nucleolus, are in each of the adjoining sections of the series.

Fig. 4. One per cent. chromic acid: safranin and orange G. Twenty-four hours. Vertical section of an egg in which fertilization is not much in advance of the preceding case.

Fig. 5. Half per cent. chromic acid: Delafield's haematoxylin. Eighteen hours. Spermatozoid caught in the slime outside an archegonium showing the kinoplasmic band extending as a wing along the forward side of the two anterior coils of the corkscrew-like nucleus.

Fig. 6. One per cent. chromic acid: safranin, gentian-violet, and orange G. Twenty-four hours. Two vertical sections of an egg. The male chromatin partly distributed in the egg-nucleus. The lightly shaded spermatozoid is in a lower focus.

Fig. 7. Half per cent. chromic acid: Delafield's haematoxylin. Fourteen hours. Horizontal sections of an egg. The large nucleolus is shaded lightly because it occurs in a lower focus than the adjacent male chromatin.

Fig. 8. One per cent. chromic acid: Heidenhain's haematoxylin. Fourteen hours. Two spermatozoids which were held in the slime outside an open archegonium. The cilia could be seen, but were not drawn because they could not be traced to their points of attachment with certainty: some were directed backward, but most of them forward. One nucleus was broken into two and the other into three pieces by the razor.

Fig. 9. One per cent. chromic acid: Heidenhain's haematoxylin. Sixty hours. Egg-nucleus from an oblique horizontal section. The large nucleolus is shaded lightly because it is behind the male nucleus.

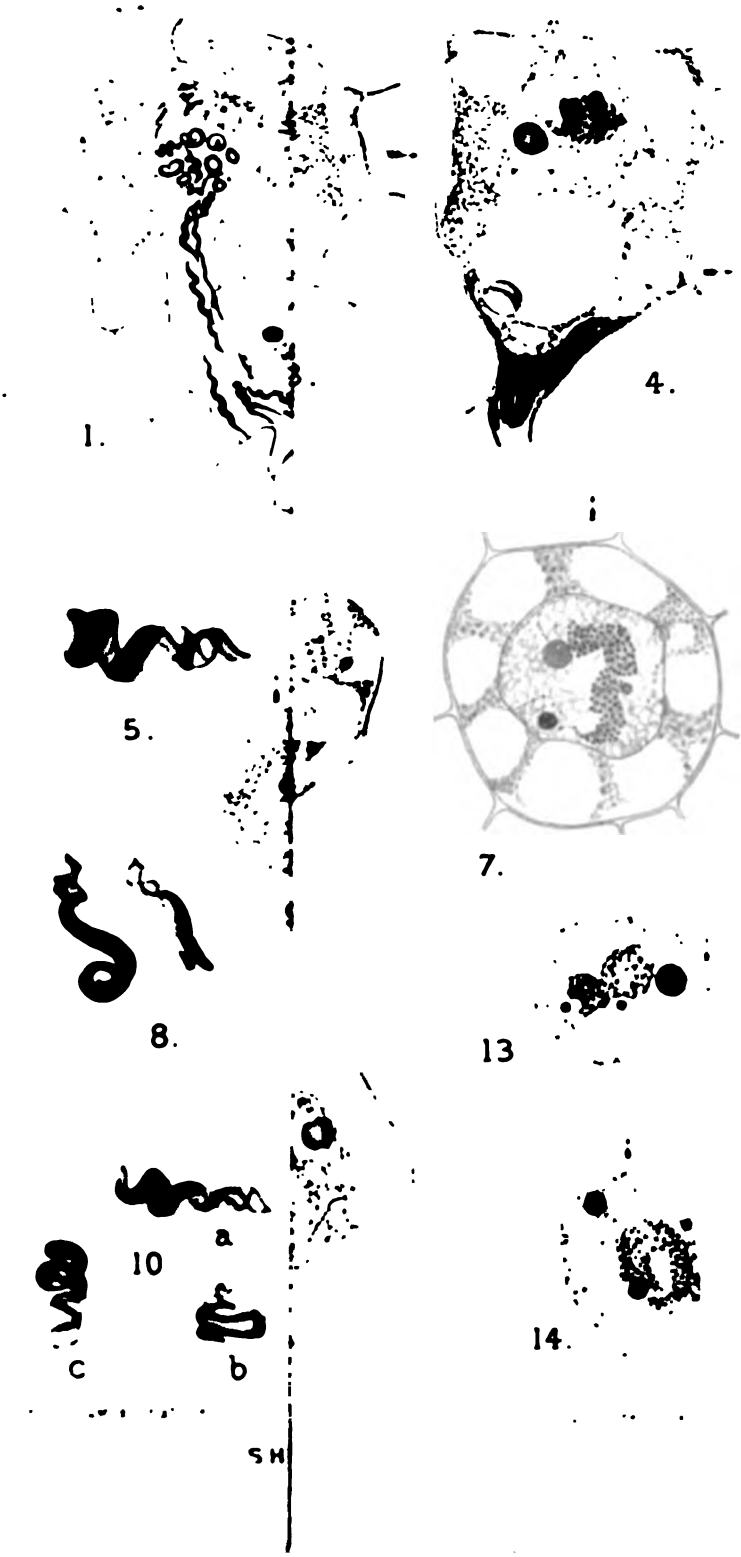
Fig. 10. Half per cent. chromic acid: alum-carmine. Twelve hours. Spermatozoids held in the slime outside an archegonium, showing the nucleus and kinoplasm.

Fig. 11. Half per cent. chromic acid: Delafield's haematoxylin. Twelve hours. Vertical section of an egg with the enclosed spermatozoid still showing the coiled spiral form.

Fig. 12. Half per cent. chromic acid: alum-carmine. Twelve hours. Horizontal section of an egg. Both the male and the female nucleus have a lininnetwork bearing chromatin bodies. The cytoplasm under most favourable illumination shows an alveolar or reticulate structure.

Fig. 13. Half per cent. chromic acid: alum-carmine. Twelve hours. Vertical section through an egg-nucleus. x marks the direction of the archegonium canal.

Fig. 14. Half per cent. chromic acid: Heidenhain's haematoxylin. Thirty-six hours. Nucleus in a horizontal section. The distribution of the male nucleus is not much further advanced than in Fig. 12 after twelve hours. The lighter large nucleolus is not in the same plane as the end of the spermatozoid.


1.
4.
5.
7.
8.
13
10
a
c
b
14.
SH

Some Thames Bacteria.

BY

H. MARSHALL WARD, Sc.D., F.R.S.,

Professor of Botany in the University of Cambridge.

With Plates XX and XXI.

I. A short colourless Bacterium, forming stearine-like colonies [1]: type of *Bacterium ureae* (Jaksch). (Pl. XX.)

THIS form is apparently not uncommon in the river; I have isolated it several times, but have only cultivated it twice through all media.

It occurs on the plates as cocci, about 1 μ diam., not motile, and grouped in pairs, or rows of four, or isolated or in heaps, and evidently developed from the breaking up of short rods 2 × 1 μ found with it (Fig. 1). On old agar-cultures the cocci alone are found; but in actively-growing gelatine-cultures the rodlets prevail and can be seen to be breaking up to cocci in all stages.

No spores have been found in any medium. The rodlets stain easily by ordinary methods—e.g. Loeffler's methylene blue, carbol-fuchsin, &c.—but they are easily decolourized by Gram's method.

[1] This is the type of Group I, referred to in Proc. R. S., Vol. 61, 1879, p. 417.

On plates at 12–15° C. it grows slowly as white, somewhat typhoid-like irregularly circular fronds, beautifully zoned and marked with radial lines. Under the ⅓ obj. these are contoufed, hyaline at the edges, and yellower in the thicker, central portion. There are no pronounced blue or green sheens or iridescence, but after a week or so the fronds appear dull (*matt*), and like thin drops of stearine with irregularly contoured edges (Fig. 2). No liquefaction even after two to three months. Plates at 20° showed in forty-eight hours as white discs, like flattened milk-drops, 1–2 mm. diam. Under the ⅓ these are yellowish, contoured and opaque, the submerged colonies being very granular.

On the third day the diam. = 2–5 mm., and with the typical opaque, stearine-like appearance.

Fourth day = 9 mm. diam., and similar appearance. On holding up to the light a beautiful shagreen-like appearance, bossed in the centre, edges like ground glass.

Sixth day = 15 mm., opaque. Indented edges to the otherwise circular frond. Traces of zones and radial structure in some.

Fourteenth day = 20 mm., zoned and radiate and with elegantly indented edges. Yellowish.

Microscopic cultures in hanging-drops of gelatine were made, but it was found to be impossible to measure the growth. The short rodlets break up as soon as division is completed, and fall apart to make the colony of cocci. Fig. 3 shows a case where the isolated rodlet (*a*) in gelatine at 20° C., at 3 p.m. had divided into two, and one of these was dividing again at 10 p.m. (*b*). At 11 a.m. next day a colony, oval, pale, and with the normal characters, had formed, measuring 99 × 85 μ in length and breadth, and several rodlets thick (*c*).

If it was ten rodlets thick, such a colony reckoned as a rectangular one would contain about $\frac{99 \times 85 \times 10}{2} = 42{,}075$ rodlets, which indicates fairly rapid rate of growth.

At the same hour (11 a.m.) the following day the colony

measured 300 x 180 μ, which, calculated as before, gives us 540,000 cocci or 270,000 rodlets.

Numerous other cultures only confirmed these results, and neither filaments nor spores could be obtained in any gelatine medium; while the minuteness of the organism rendered futile all my attempts to directly observe its growth in liquid media.

During active growth the rodlets 2 x 1 μ prevail, but as the colony ages these all break into cocci.

Stab-cultures at 12–15° show up in three days, but the development is slow. Yellowish-white dots appear in the tunnel, and a thin, dull, ground-glass-like frond above. This is hard and tough, like stearine, and difficult to scrape off. In fourteen days the surface is nearly covered, the frond with beautifully indented margins and radiate structure. No further growth in the tunnel. The matt appearance is due to a rough shagreen-like surface (Fig. 4).

At 22° the development is equally good on acid or alkaline gelatine, and on the third day the characteristic matt frond appears above. The dots in the tunnel coalesce a little, indicating feeble growth. No trace of liquefaction. In old cultures the gelatine is slightly brownish-tawny above, and the colony has a faint greenish yellow tinge. The stab is sword-shaped. No trace of liquefaction even after three months' culture: the yellowish white, thin, waxy, shagreened growth just covers the top of the gelatine, and has delicate fimbriate margins. Colonies submerged in the gelatine exhibit no growth.

Streak-culture at 20° spreads fairly quickly, as a white, thin, matt film, like stearine or ground-glass, in forty-eight hours. In seven days nearly the whole surface is covered with a beautifully frondescent very thin film, spreading from the thicker streak, flush with the surface, greyish-white like ground-glass (Fig. 5). No sign of liquefaction even after two months. Edges very thin and fern-like.

On agar at 30–35° a faint streak and one or two thin spots appeared in two days: these hardly increased in eight days.

On keeping at lower temperatures a tough paste-like dirty white patch slowly spread in a month.

At 22° white granular discs appeared in twenty-four hours, and coalesced to a thin spreading yellowish white film in two to three days. Slight white deposit in drainage. In a week there was little progress: the individual colonies are thin fronds like those on gelatine plates, but remain small. They are often polygonal where their edges touch, and give a curious mosaic-like or scaly look to the growth (Fig. 6). Under the lens these resemble the scales of a Turbot. In other cases the film is continuous.

Potato at 34° gave no results in a week, but at 22° a dull, white yellowish streak appeared in twenty-four hours, with the peculiar stearine-like look of the gelatine colonies. In forty-eight hours this was thicker, dry, yellowish grey, and in four days had crenate and somewhat mesenteric edges. The colour darkened with age—buff, and like dull wax. In a week this consisted of rodlets $2 \times 1\ \mu$, breaking to cocci $1\ \mu$ in diameter. Old cultures at 34° showed a slight growth after one month at lower temperatures. After two to three months the growth turns umber-brown.

Broth at 25° formed dense flecks above in twenty-four hours, which easily shook down. In forty-eight hours a dense greasy-looking flocculent veil above, falling at the slightest shake: abundant deposit. These flocculent veils are renewed, the intermediate liquid remaining clear. The very abundant flocculent deposit acquires a slightly buff-white tinge with age.

Milk at 25° showed no change in a month, beyond a distinctly acid reaction. On keeping three months still no change observable.

Glucose at 30° formed white flocks below in twenty-four hours, and this deposit increased in two to three days. But no bubbles or turbidity resulted. At 25° the flocks and deposit were slightly more abundant, and resembled the broth cultures. No turbidity. The deposit slightly yellowish, but not very abundant even in three weeks.

Urine at 25–30° gave very slight traces of turbidity in

twenty-four hours, and subsequently a few bubbles formed, and granular flocks were deposited. No definite turbidity, and no increase in three weeks.

The most likely form I have been able to trace resemblances to is Jaksch's *Bacterium ureae*, which may be distinct, as he believes it is, from Pasteur and Van Tieghem's *Micrococcus ureae*.

It agrees with Jaksch's form in the size of the rodlets, the general characters of the plate cultures, and particularly in the dull (*matt*), ground-glass appearance. The stab-cultures also agree fairly well, though I have never detected the smell of 'Häringslake' noted by Jaksch.

The growth is described as 'ungemein langsam[1].' Of course this is very indefinite: in my form the growth is slow but not uncommonly so. The general behaviour as to temperature agrees, so far as can be gathered from the meagre information to hand.

I prepared tubes of Jaksch's fluid as follows—per 1 litre water:—

$\frac{1}{16}$	gram.	$Mg\,SO_4$.
$\frac{1}{8}$	„	$K\,H_2PO_4$.
5	„	Rochelle Salt.
5	„	Urea.

In this perfectly clear liquid the organism grew very slowly, forming grease-like flecks and films on the surface and a very white deposit. Better at 25° than at 35°. No odour could be detected, and it is pretty evident that if this is Jaksch's form, it grows but feebly in the liquid given.

M. ureae seems to differ from the rodlets chiefly in growing more quickly and at higher temperatures, and in the cocci—which may also be in pairs or tetrads or chains—and in the occasional formation of zoogloea.

The stearine-like plate-cultures are very suggestive, and the stab-cultures agree well, except that I have not noticed the odour described.

[1] Zeitschr. f. Phys. Chemie, Bd. 5, p. 395.

In both cases we are devoid of information as to the behaviour in other media than gelatine and urine or Jaksch's fluid, so that it is impossible to be sure of the identity of these forms.

There are some distinct resemblances also to Zimmermann's *M. concentricus*[1], but he does not note the dull stearine-like appearance which is so striking in all my cultures.

As Tataroff[2] himself remarks, his 'Perlmutter-glänzende Diplococcus' may be *M. ureae*, and the resemblances are noted.

The following tabular summary gives the salient characters of this Bacterium.

Habitat.	Not uncommon in the Thames.
Morphological characters.	Cocci about 1 μ, not motile, single or grouped in pairs, rows of four, or in heaps, and formed by breaking up of rodlets 2 × 1 μ.
Plates.	At 12–15° C. forms slowly growing white, typhoid-like, irregularly-circular, contoured, zoned, and radially-marked fronds: edges hyaline, centre yellowish. Become dull, matt, and like thin stearine drops.
Stab.	12–15° yellowish white dots in the tunnel, and a thin frond-like ground-glass above. This is hard and tough, and matt, as in plate colonies. Quicker at 20–22° C.
Streak.	At 20° grow fairly rapidly as a white, thin, matt film, nearly covering the whole surface in a week. The streak thicker.
Agar.	At 30–35° grows slowly as a dull white and dryish layer. At 22° the characteristic white waxy matt film forms more rapidly. In some cultures isolated colonies form on the surface and coalesce to form the film, as polygonal Turbot-scale-like mosaic.
Potato.	No growth at 34°. At 22° a dull, yellowish-white, waxy, dry streak-like stearine, darkening to buff, and after some weeks to umber.
Broth.	At 25° forms dense floating flecks and greasy white films, which fall as an abundant deposit, eventually buff-white. The films are renewed and liquid remains nearly clear. The greasy films adhere to sides of tube above.
Milk.	No change at 25° beyond acid reaction.
Glucose.	No turbidity, but white veils and flocks form and fall as in broth, but less abundant. Better at 25° than 30°.
Urine.	At 25–30° a few bubbles and granular flocks only, and only slight traces of turbidity.

[1] Die Bakterien unserer Trink- und Nutzwässer, Chemnitz, 1890, p. 86.

[2] Die Dorpater Wasserbacterien, 1891, p. 71.

Jacksch's liquid. Slow growth and greasy flecks, falling as a very white deposit. Better at 25° than at 35°. No odour.

Pathogenicity. Not pathogenic to guinea pigs, according to Professor Kanthack's report[1].

After remaining from May 28 to June 8 of the following year, i.e. over twelve months, it was found quite easy to revive this form from an agar tube. Good plate-cultures resulted in four days at 20–22° C., and the colonies were quite characteristic. Further cultures in gelatine, agar, potato, broth and milk tubes confirmed this, and the results at 25° and 35° respectively were as above.

This form must therefore be regarded as a very constant and persistent one, in marked contrast to many of the others I have had to deal with.

II. A colourless capsuled Coccus or Bacterium[2]. (Pl. XX.)

An interesting form, isolated and cultivated through all stages at least twice from the river in the autumn, was one which occurred on the plates as small, short, oval, non-motile rodlets over 1 μ long by 0·75 to 1 μ broad, and invested by a tough dense zoogloea or capsule, which occurred round the groups of dividing rodlets—then biscuit-shaped—as well as round individual cocci or rods.

If rapidly stained by Gram's method the capsules are decolourized, and the rodlets coloured: but they are easily decolourized. The stained rods = 1 × 0·6 μ to cocci about 0·6 μ. The capsule = about 6 to 10 μ.

On plates at 12–15° C. the colonies are white, porcellanous, shining discs or fronds, with a central spot and faintly zoned. To the unaided eye the colonies look bluish-white and translucent if held up, the zone or zones appearing yellower: the zones sinuate in agreement with the indented

[1] I have to thank my colleague, Professor Kanthack, for kindly examining a number of these bacteria for me in respect of their pathogenic properties.

[2] Referred to as the type of Group XI in Proc. R. S., Vol. xli, p. 420.

margins. Under the ⅓ obj. the whole colony looks yellowish, granular, and gradually becomes more opaque in the centre as the frond thickens, the margins thinning out and paler.

The microscopic examination also shows the colonies marked by irregularly and curiously contorted lines and streaks (Fig. 1 *a*), and scattered sets of brighter, rounded, sausage-shaped and vermiform areas. In the older colonies these are less visible in the centre, owing to the opacity as the colony thickens, but the zoning is found to be principally due to these brighter areas nearer the margins.

The submerged colonies are yellow, granular, opaque, and irregularly lobed like a complex glandular acinus or salivary gland (Fig. 1 *b*, *c*).

As the colony ages—three to four weeks—the white becomes tinged with a tawny hue, and a tendency to soften and sink into the gelatine is evident.

Closer examination with a higher power shows that the bright vermiform and rounded areas are dense zoogloea masses embedded in the granular matrix of the colony, and that the glandular submerged colonies and the dark central part of the emerged ones are simply dense and irregularly-lobed zoogloeas containing the cocci and short rodlets, the rest of the colony consisting of irregularly and closely-crowded escaped cocci without any evident capsules (Fig. 1 *d*).

These imbedded zoogloeas are so obviously similar to Cohn's *Ascococcus Bilrothii* that I referred to them throughout my notes as *Ascococci*, but—though unfortunately we do not know the size of Cohn's form—the cells seem to be larger, and they are certainly not permanently cocci, as will be seen later.

At 20° the colonies were visible in twenty-four hours as minute grey points, yellowish and granular under the ⅛. A higher power (Zeiss D) showed them already lobed and capsuled. On the second day they form white opaque irregular circles 2 mm. diam., and like milk: under the ⅓ the submerged colonies are lobed and glandular, the emerged

ones form discs, with the Ascococcus-like groups imbedded.

On the third day they are like irregular milky drops, too thick to show structure.

Stab-cultures at 12–15° form a wet, glistening, thin white frond above and yellowish-white, dense, dot-colonies in the tunnel. In a week the frond has nearly covered the surface of the gelatine, and is depressed in the middle, slightly sinking into the gelatine; while the colonies along the tunnel enlarge and tend to radiate into the surrounding gelatine. The sinking goes on until the frond lines the sides of a distinct funnel, devoid of liquid however; and the submerged colonies form cloudy outgrowths and widen the tunnel. The sinking and softening of the gelatine continue, and are very decided in a fortnight to three weeks (Fig. 8).

At 20° C. the phenomena are similar but quicker. In five days the softening of the gelatine is pronounced, the submerged colonies confluent; and a good funnel with signs of liquefaction and running are evident in ten days. The growth is equally good—or even a little better—in slightly acid gelatine, as compared with slightly alkaline.

The growth is easily removed by the needle, but does not lift as a whole membrane, and is firm and waxy or slightly slimy. Even after ten weeks there is no real liquefaction of the gelatine, but the cloudy white growth was penetrated far in.

At 20 in sugar-gelatine, a milk-like spreading drop formed above, and a considerable confluence and growth in the tunnel in three days.

Streak-cultures at 20° show a dull, translucent, white growth, yellowish if held down, bluish by transmitted light, thin at the margins, spreading slowly, and softening the gelatine in eight or nine days, and beginning to sink along the axis (Fig. 9).

In a month a deep spoon-shaped scooping has occurred, in which the cloudy white growth floats in viscid softened gelatine.

Agar. At 20° C. a copious, thick, spreading, glistening, pure white streak with iridescent edges, extending to a frondescent film all over in twenty-four hours. In three days a thick, shining, translucent, waxy, yellowish-white layer. On the fifth day this is a wrinkled membrane, and a white wrinkled veil and precipitate are seen on and in the liquid of drainage.

This glassy-looking membrane is tough and lifts as a whole, and the microscope shows it as a dense zoogloea, with rodlets breaking up to cocci in Ascococcus-like masses. Staining with acetic acid and dahlia-violet shows that the capsules enclose both single rodlets and colonies (Figs. 3 and 10).

At 34° C. the growth is similar, but less rapid. In four days the gum-like, translucent membrane is formed, but even in eight days it had not covered the surface.

In strong growths the Agar is evidently diminished in a few days, serving as food-material. After a week or two at low temperatures the corrugated membrane is renewed on the stripped Agar.

Potato. In forty-eight hours, at 22° C. a thin, wet, spreading, glistening film is formed, white at the thin fimbricated spreading margins, very pale yellow inwards, and with a greyish cast where thickest in the centre. About the fourth day the thin white margin disappears, and the whole patch is wet and slimy (Fig. 11).

The microscope shows that the wet sulphur-white to yellowish-grey slime consists of rods 1·5 to 4 $\mu \times 1\ \mu$, motionless, embedded in a tough slime which draws into long strings on the needle.

At 22° alcaline potato is an equally good medium with normal, the colour of the copious whitish slimy growth being perhaps less grey and more sulphur-yellow in hue.

Artichoke at 25° C. A white irregular patch was formed in two days, and spread all over as a white film on the fourth day, after which no further growth was noted, even in fourteen days.

Carrot at 25° gave good results. In twenty-four hours a rapidly-spreading gum-like layer formed, and extended all over as a wet, thin, watery layer in forty-eight hours. No change on the fourth day, and matters were the same at the end of a week. In fifteen days the tubes were discarded—no further growth.

Turnip at 25° gave no certain results in four days, and even after a week no growth was observable. Kept for three weeks—no further results.

Broth. No growth at 35° in three days. The liquid remains perfectly clear. At 25° C., however, the broth is turbid in twenty-four hours and with a dense precipitate, which is white in three days. Even after a fortnight the liquid is still densely turbid, and a copious flocculent precipitate has fallen.

Glucose at 25° showed no trace of activity in three days. A tube put in at 30°, when the temperature was falling to 25°, gave a slight turbidity in three days, and traces of white precipitate, but no fermentation visible; this remained the same on the ninth day. In other cases no results were obtained in two or three weeks at 25° C.

Milk at 25° C. No change to third day, but in fifteen days a thick custard is formed, and the tube can be upturned. In eighteen days the casein falls. The reaction is acid. No signs of solution in five weeks at 25° C.

Urine at 22° gave a slight turbidity in five days, with traces of a ring, but no signs of further growth.

Pathogenicity. This form was kindly examined for me by Dr. Lazarus Barlow and gave pathogenic results. A guinea-pig inoculated in the peritoneal cavity with about 10 c.c. of a four days' old beef-broth-culture died in twenty-three hours.

On examining Dr. Lazarus Barlow's preparations, I found them exactly to type. That from the peritoneal fluid showed the capsules, faintly but distinctly, but in the others they were almost invisible. It should be noted that no information had been given when the tubes were handed on, and so no

attempt to bring out the capsule had been made (Figs. 4 and 5).

Professor Kanthack also found that this form is pathogenic to guinea-pigs, though as he was working with smaller doses, the results were not so fatal. Inoculation into the thigh produced a large swelling, intra-peritoneal injection made the animal very ill for a day. In both cases, however, the guinea-pigs recovered. The experiments gave the same results on repetition. It is worthy of note that the form sent to Professor Kanthack had been much longer in culture than that sent to Dr. Lazarus Barlow.

On being revived on July 13 from an Agar-culture which had stood since May 13 of the previous year, i. e. fourteen months, the plate-cultures gave normal colonies, showing the characteristic zoogloeas embedded in the mass. On potato also the cultures showed the characteristic yellowish pasty growth with a broad white marginal area on the brownish-grey potato, and the other cultures were normal, and no question could arise as to identity. Even the custard in milk was developed in fourteen days at 30° to 35° C.

All attempts to revive another culture failed.

De Toni and Trevisan[1] have attempted a classification of capsuled micrococci along the following lines:—

They group all the forms under the head of Ascococceae. Then they cut out Winogradsky's *Amoebobacter*, chiefly on account of the amoeboid movements and arrangement in series. The remainder are divided up, first according as the 'capsule' is *general* and around whole colonies, or *special*, i. e. around each individual coccus. Further subdivisions depend on whether the 'cysts' or 'capsules' are lamellated or not, whether the colonies or families consist of few or many individuals, whether the divisions are in one or more planes, and so on.

It seems difficult to accept the details, but no more consistent attempt is to hand, so far as I am aware.

[1] Sylloge Schizomycetum, p. 1035.

There are nine genera, as follows:—

Lamprocystis, which includes only Lankester's *Bacterium rubescens*, with its numerous (real or assumed) synonyms.

Ascococcus, again confined to one form—Cohn's *A. Billrothii.*

Bollingera, comprising two species of *B. equi* (*Micrococcus ascoformans* of Johne, *M. botryogenus* of Rabe) and *B. Vacchetae* (Trev.).

Cenomesia, also with two species—*C. albida* and *C. lilacina*, both from sulphur waters.

Thiocystis, again comprising two forms — Winogradsky's *T. violacea* and *T. rufa*, both from sulphur waters.

Thiothece, including Winogradsky's *T. gelatinosa*, from sulphur springs.

Leucocystis, with only Schroeter's *L. cellaris*, found in caverns, &c.

All the above are regarded as having a general capsule, common to whole colonies or families: the following are devoid of this, but each coccus has its own special investment:—

Chlamydatomus includes the two species: *C. Beigellii*, first described by Beigel as a *Gregarina* found on hair, and *C. cellaris*, found by Hansgirg in cellars.

Gaffkya includes four species: *G. grandis*, the *Microcoque des reins et des ulcères syphilitiques de la peau* of Babes and Cornil.

G. tetragena (Gaffky), *Micrococcus tetragenus*, found in phthisical sputum.

G. Mendozae (Trev.), *M. tetragenus mobilis ventriculi*, a motile form which gives an odour of skatol in cultures.

G. Archeri (Trev.), Archer's *Black Micrococcus*, a deeply pigmented form found on potatoes.

But these are not the only micrococci described as having these 'capsular' investments, as the following list shows:—

Micrococcus of Bovine pneumonia, Poels and Nolen, from the lungs of cattle infected with pleuro-pneumonia, and resembling Friedlander's bacillus in many respects.

Diplococcus of Horse pneumonia (Schütz), a similar but imperfectly described form.

Haematococcus Bovis (Babes); *Pseudodiplococcus pneumoniae* (Bonome), indistinguishable from *M. pneumoniae crouposae* except in its growth at lower temperatures; *M. ureae* (Pasteur); *M. luteus* (Cohn.); *M. viticulosus* (Katz), are other species described as capsuled or forming investing zoogloea masses.

I am unable to refer my Thames form to any of the foregoing with certainty, and am inclined to suggest that it should receive a name as a new 'species.'

From a Petri-dish, in which a plate-culture had been made from a drop of water impregnated by shaking up a zoogloea-mass grown on Agar, I removed a little of the gelatine-film with a loop, and transferred it to a culture-cell, suspending it from the cover-slip as for a hanging-drop culture.

The plate-culture had been going twenty-four hours at 20°, and the colonies were just visible—hardly so without a lens—and my idea was to watch the behaviour of a rodlet at the thin margin of a colony.

To do this, however, it was necessary to raise the temperature of the culture chamber just sufficiently to soften the gelatine and make it spread a little, for no matter how carefully one prepares such a culture as the above, the play of lights reflected and refracted at the conchoidal fractures of the solid splinter of gelatine interferes seriously with observations under high powers.

Consequently it was necessary to warm the whole to nearly 25° C., and then let the minute-drop solidify again.

This was done, and several well-isolated rodlets were now found near the margin of the colony and clear of it. I now focussed a pair, lying close together but sufficiently apart for distinct observation: their position was fixed by means of the micrometer, and they were drawn at 10 a.m.; the temperature being 21·5° C.

Their behaviour at subsequent periods of observation is given in Figs. 16 *a-f*. At 10.20 each had divided, though the two halves were still joined: at 10.35 they were free, and now there were four rodlets in place of two (see Fig. 16 *c*). At 11.10 a left-hand rod was dividing, as shown by its biscuit-shape, and at 11.40 there were six rodlets; at 12.20 a rod below, to the right, was dividing, and by 12.45 there were eight rodlets.

Now it was evident that in the successive divisions the sister-halves were not equally capable of dividing. The question arises whether this is due to position, or some other cause. I am strongly inclined to regard it as due to position; in each case the new divisions occurred first in cells *nearest new territory*, i.e. advancing away from the colony into unexplored gelatine.

The above observations had now to be interrupted, and on resuming them at 3.20 p.m. a startling discovery was made—*all the free bacilli were in active swarming movements.* The temperature had slowly risen to 23·5 and remained there, and the gelatine-drop had absorbed a great deal of water: these factors, taken with the liquefying power of the colony, explain why the drop was now liquid.

But the swarming was an unexpected phenomenon. I had got over my surprise at the isolated rodlets, above described, showing no capsules, because earlier examination of the gelatine colonies showed that not all the cocci or rodlets are capsuled. Hitherto, however, they had shown no signs of movements. The obvious suspicion arose that an intruding swarmer had got into my hanging-drop.

That was not the case, however, as the following observations show. As we have seen, the temperature had been slowly rising all the morning, as follows:—

10·0	a.m. temperature	= 21·5
10·20	„	= 22
10·35	„	= 22·25
11·10	„	= 22·5
11·40	„	= 22·5
12·20	„	= 22·75
12·45	„	= 23·25
3·20	„	= 23·5

And I allowed this rise to go on. The numbers of swarmers increased enormously, and I suspected this was not due merely to the rapid division of those already in motion, but that the increase was partly due to reinforcements from the colony of resting forms.

After some search—principally due to the difficulty of focussing now the drop was enlarging—I got a very typical capsule enclosing six rodlets under observation at 3.40. The temperature was 24·5°, and remained there. But the rodlets inside this cap were no longer quiescent: they were slowly moving, tumbling over one another within the hyaline prison of the capsule.

Numerous free swarming rodlets were now in the neighbourhood, and one saw here and there groups of about six to ten of apparently free ones moving about each other, gliding and tumbling one over the other in the same way as those imprisoned in the capsule referred to.

This capsule was kept under observation from 3.40 to 4.40, and notes made at 3.55, 4.15, and 4.30.

The slow swarming at 3.40 became more and more active as time went on, and at 4.15 was as active as in the apparently free swarming groups around, but the enveloping capsule was now swollen, and so transparent that it could only be known to be there by the limits its presence placed to the swarming movements of the imprisoned bacilli. At

4.30 the diffluent walls had softened and dissolved, and the imprisoned swarmers escaped, and were swimming about as actively as any of the other free rods.

These observations were confirmed several times—as soon as the temperature rises to about 23·5 to 25° active swarming begins.

In some cases a pair of recently divided rodlets behave in a peculiar manner, and this I have seen not once, but several times. Each is capable of movement on its own account, but in some cultures (gelatine) the newly-separated rodlets separate by backing away in a straight line, and then come together again, end on, and remain a few seconds as if they had never separated at all. Thus, in Fig. 13 I have sketched the relative positions of one of these pairs at four stages of their oscillations. At first they were closely applied pole to pole in a straight line (*a*), then they suddenly darted asunder (*b*), till separated by about three times the length of either: after a few seconds they flew together again (*c*), and then again flew apart (*d*); and this went on for at least half an hour.

It is quite a common event to find the rodlets swarming in this way, though the pairs do not invariably approach and recede in the same straight line. It was noticed that in one and the same case the movements in either direction · separation or flying together—might concern both, or only one, or sometimes one and sometimes the other, and by no means equally. If we call the rodlets *A* and *B*, sometimes they darted apart five or six times the length, equally distant from the point where their poles joined, and next time *A* would dart off and leave *B* quiescent, or *A* would move twice or three times as far as *B*: similarly on darting together. I could find no rhythm about this phenomenon, and do not understand its meaning. At one time I thought the darting together might be due to an elastic cilium which they were tugging at, but it seems improbable.

In most cases, however, the swarming is in and out irregularly when several are concerned, and it seems to

depend on the temperature. Fig. 14 gives a case where the single rodlet at 10.25 p.m. had divided at 11 p.m., and the two halves separated at 11.5 p.m.: these two oscillated away from and towards one another as above described, and went on doing this and dividing through the night, and at 7 next morning had developed into a colony about 25 μ in diameter, and containing probably 10,000 to 15.000 of the short rodlets, all swarming actively, in the circumscribed space of their own gelatinous investment.

If the temperature does not rise beyond about 20° the colonies are developed without any swarming. Thus Fig. 15 shows a case where a rodlet was fixed at 4 p.m. in 10% gelatine and remained at 15° C. through the night. Next morning at 8·15 it had formed a small colony about 3 μ in diameter, and consisting of eight to ten rodlets, so far as I could make out—possibly twelve. The temperature was then allowed slowly to rise to 20–25° C., and at 3 p.m. the lobed colony of quiescent short rodlets and 15–16 μ in diameter, shown in Fig. 15 (*c*), had formed. At 8.30 p.m. the whole colony was in active swarming, but next morning was quiescent again.

Numerous attempts to cultivate this form further were made without success.

III. Rose-pink Micrococcus: Type of *M. carneus*. (Zimm.) (Pl. XXI.)

A very pretty rose-pink form was isolated several times and studied during the winter of 1894–95, when it seemed fairly common. It is by no means one of the more frequent forms in the Thames, however. I was for some time puzzled by it, for at one period its alliances seemed doubtful. It occurs as spherical cocci of variable sizes, from 0·5 to 1·0 μ, or even occasionally up to 1·5 μ or nearly so, in diameter, in irregular botryoidal groups, and perfectly quiescent.

It stains easily, and well-stained specimens may show a darker more or less central spot, and a paler halo round

the cells. Specimens in water sometimes seem to be distinctly vacuolated, or even to have granules in them, and some of these characters at first led me to suspect its being an extremely minute yeast-form—for instance, the vacuolations, the paler halo, and the grouping—but I have been able by cultures to determine that this is not so: it is a true Schizomycete. The fact that it does not ferment glucose solutions is, so far as it goes, evidence against the yeast view; but of course it is far from conclusive, since plenty of yeasts do not ferment sugars. In the absence of any proof of budding I considered this form as probably a *Micrococcus*, and the occurrence of diplococci and rows of nearly or quite equal-sized cocci point to the same conclusion.

On cultivating it at 19–20° in broth-drops under the $\frac{1}{12}$th immersion it proved to be a *Sarcina*-like *Micrococcus*[1], which divides in all three directions, but the progeny frequently partially separate later on, and only remain united in zoogloea-masses, and so form irregular botryoidal groups of cocci each 1–2 μ in diameter. The high refrangibility of the gelatinous zoogloea investment makes it impossible directly to see the actual act of division, but enough evidence was obtained (see Figs. 8 and 10) to determine the nature of the organism.

After being sown about twenty-four hours, the cocci are found dividing very regularly in the *Sarcina*-form (Fig. 8), but in the course of another twenty-four hours the cocci partially separate as they rapidly divide, and, rounding off, remain agglomerated in the characteristic grape-like manner shown in Fig. 8 *d* and Fig. 9. As time goes on, the separation is more and more complete, and isolated cocci and diplococci are common in the drop.

The series figured in Fig. 8 (*a* to *d*) will show this. At 11.50 a.m. a group of three *Sarcina*-masses was isolated (*a*) and watched: at 2.30 p.m. the *Sarcina*-divisions had increased as seen in (*b*), though it was impossible to accurately

[1] The type of Group XVII in Proc. R. S., Vol. xli, p. 421.

count the cells. The group had rotated through about 180° in the interval.

At 4 p.m. the further development seen in (*c*) had taken place, and signs of loosening of the individual cells were evident, and at 9.50 p.m. the group was a rapidly increasing botryoidal mass as shown at (*d*). Next morning it was a loose mass of groups like Fig. 9.

The best series, however, is the one in Fig. 10, where I traced the whole course of development under the $\frac{1}{20}$th immersion. The gelatine-drop was prepared at 5 p.m., and after allowing time to solidify, &c., the single coccus drawn in Fig. 10 (*a*) was fixed at 5.55, t = 20° C. At 8.5 this had grown to the biscuit-shaped figure shown at (*b*); and at 11.40 p.m., the temperature having fallen to 19° C., there were four cocci in focus (*c*). Whether growth had occurred in the plane at right-angles to the paper I could not with certainty determine, but was of opinion that it had. During the night the temperature fell to 16° C., but was at 18° by 9.10 a.m., when nine cocci were clearly visible (*d*), and certainly some existed in the depth, but I could not focus down to them.

By noon, growth was rapidly advancing, and two groups of four, one of two, and some behind were visible (*e*): the temp. = 19° C.

At 2.10 (t. = 19°) the group was loosening (*f*), and this went on as the growth and division rapidly advanced (*g* = 4 p.m., t. = 21°), till at 9 p.m. (t. 22°) there was a mass like a bunch of grapes (*h*).

Plate-cultures at 12–15° C. show slowly-developing, raised, dry, rose-pink points, which even after three weeks are not more than 1–2 mm. in diameter, and do not as a rule liquefy. In a week the submerged colonies, under the $\frac{1}{3}$rd objective, are irregular, roundish, dull-pink and granular; while the emerged ones are prominent, rose-pink, opaque drops, showing a deeper centre, and a paler granular zone around. Even after two months the rose-pink, slightly sunk, projecting points are not bigger than in three-weeks' plates (Figs. 1–3).

Under the $\frac{1}{3}$rd objective the older emerged colonies show

as granular discs with a colourless margin and deeper centre, others are distinctly zoned, pink, and considerable variation in the depth of colour occurs, from pale-brick red to lavender-tinted rose-pink.

Gelatine streak at 20°. In twenty-four hours the growth begins as a dry, pale lavender, tinted rosy streak, much the colour of almond petals. In a fortnight it has a curious appearance of striping, like fresh-cut muscle under a lens. The colour gets more like sealing-wax at the thickened base. The transversely striped appearance—due to ridges—seems a constant character. In the course of a month or more it slowly liquefies, and in six weeks seven-eighths of the gelatine is quite liquid.

Stab-cultures at 12–15° show small dots in the puncture-line in five days, and a protuberant dry pink button above. The colour deepens to plum-pink as the button widens, and in eighteen days no trace of liquefaction occurs.

At 20° the growth is similar (Fig. 4), but traces of sinking are found in five weeks; in six to seven weeks the gelatine is liquefied half-way down, and even more.

It requires three months or more to complete the liquefaction to the bottom.

Agar. In forty-eight hours at 25° a dryish rosy streak of isolated and conjoining raised dots. In three weeks confluent to a shining, pasty, rose-pink, broad streak, with thicker axis, and flattened, radiately striated mesenteric and indented margins (Fig. 5). Consistence pasty. The hue is a lavender-tinted rosy pink, much like almond petals.

After being in culture some time on Agar at 25°, numerous minute dot colonies are formed, hardly showing trace of pink in six days: faint pinkish deposit. The growth at 35° is still more faint, minute pink dots appearing in ten days.

Potato. At 20–22° forms a pink, rather moist, spreading layer in three days, which in five days becomes almost vermilion, thin, and spread all over. The colour is very peculiar; perhaps carmine is the nearest hue (Fig. 6). The growth on alkaline potato is extremely slow, or even *nil*.

Normal potato at 35° shows very slight growth in forty-eight hours. Little progress in four days to a week: merely a few extremely minute red spots in a watery film.

Carrot at 25° gives a thin and very poor pinkish-white growth in fourteen days.

Artichoke. No growth in fourteen days at 25°.

Turnip. No growth in fourteen days at 25°.

Broth. No growth at 35° in a week, nor after a month's subsequent keeping at ordinary temperatures. At 25° a faint pinkish deposit in three days, but no turbidity. In a fortnight the deposit is increased—granular and flesh-pink: no turbidity or other change. The pink slowly deepens in hue.

Milk. At 25° showed no change in fifteen days beyond traces of pink in a small white deposit. This had not increased by the third week, when the liquor was faintly but distinctly alkaline in reaction: no other change, but in the course of two or three months there are traces of peptonization without coagulation.

Glucose at 25°. Showed no growth in fourteen days. Not proved to be pathogenic for guinea-pigs according to Professor Kanthack. The results are doubtful.

The following pink, non-liquefying micrococci and yeasts are on record.

Micrococcus cerasinus siccus (List[1]) is a very minute form, 0·25 to 0·32 μ in diameter, found in water, but growing best at high temperatures—e.g. 37° C.—and not doing well on gelatine. It is interesting to observe that this form is also noted as resembling a Torula in some cases, but it is incapable, according to Adametz, of inducing fermentation. The description to hand is very meagre, but the size, temperature, and other characters seem different from those of the Thames organism.

M. carneus (Zimmermann)[2]. This form, found in the Chemnitz water-supply, presents some striking resemblances to the Thames one. The cocci average about 0·8 μ in

[1] Eisenberg, Bakteriologische Diagnostik, p. 34. [2] Zimmermann, l. c., p. 78.

diameter, and are arranged in irregular botryoidal clumps. It grows best at ordinary temperatures, and poorly at 30–33° C. The growths on Agar and Potato are strikingly similar to my results, but there are minute differences in the description of the plate-colonies, possibly due to differences in the temperature of our cultivations. Lustig[1] describes a red form (*Coccus ruber*) which Maschek found in water, and which he regards as probably identical with Zimmermann's species. The differences in the two descriptions are nearly, if not quite, as great as those between Zimmermann's account and mine, only Lustig gives too few particulars (e.g. as to temperatures, &c.) for a decisive judgement.

Another red Micrococcus is Flugge's *M. cinnabareus*[2], also found in water and air. Excepting that the cocci are described as 'large,' and frequently in pairs or in tetrads, and that the plate-colonies are red-brown under the low power, there is nothing in the short diagnosis to separate this form from the above, and we may well suspect that they are one and the same form, for the naked-eye colours of Flugge's species agree very well. Of course much depends on what 'large' means in his diagnosis[3].

Macé[4] describes under the name *M. roseus* (Flugge) a common air-form, in twos, threes or tetrads, with flat faces, about 1·4 μ along the greatest diameter. It does not liquefy, but the description is too meagre to make much of. Macé also points out how similar these forms appear to be, and remarks that the form termed *M. cinnabarinus* of Zimmermann cannot be distinguished from Flugge's *M. cinnabareus*.

This *M. roseus* of Flugge must however be distinguished from the *M. roseus* of Eisenberg referred to below, as well as from the *M. roseus* described by Maggiora[5], a non-

[1] Diagnostik der Bakterien des Wassers, p. 40.

[2] Flugge, Die Mikroorganismen, 1886, p. 174.

[3] Macé, Traité pratique de Bactériologie, p. 335, gives 0·9 μ, which would strengthen the force of the above.

[4] Macé, p. 334.

[5] Giorn. d. Soc. ital. d'igiene, Anno XI, 1889, p. 356, No. XXII.

liquefying form, 0·6 μ in diameter, associated in irregular glomeruli, and forming a pale rose pigment.

Mention may also be made of *M. agilis* (Ali-Cohen [1]), a motile form, 1 μ in diameter, and which sometimes liquefies slightly after a long time: a pink layer is formed on Agar and potato.

In addition to the foregoing non-liquefying forms, may be mentioned a series which liquefy the gelatine:—Bumm's *Diplococcus roseus* [2], a liquefying air form; *Sarcina rosea* (Schroeter [3]), also a liquefying aerial form; *M. roseus* (Eisenberg [4]), a slowly liquefying form found in sputum.

Sarcina mobilis [5] (Maurea), said to be motile (?), liquefies, and will not develop on potato.

Finally, reference may be made to *Bacillus prodigiosus*, which is often termed *Micrococcus prodigiosus*, owing to the shortness of its rodlets: this seems identical with *M. haematodes* described by Zopf [6] as the form concerned in bloody sweat.

The resemblances to Zimmermann's *M. carneus*, which he regards as probably identical with Maschek's form [7], is so marked that only one point of importance indicates lack of identity. This is as regards the mode of division. Zimmermann says (l. c. p. 78) the divisions occur in one direction only, but I find the divisions occur in all directions, and that in certain stages the groups resemble a *Sarcina*. It is an interesting point that Maschek's form (I quote from Lustig, l. c., p. 40) presents the same similarity to a *Sarcina* that mine does, and we have seen that Zimmermann regards Maschek's

[1] Central-bl. f. Bakt., 1889, VI, p. 36.

[2] D. Mikroorg. d. gonorrhöischen Schleimhauterkrankung, 2. Ausg., Wiesbaden, 1887 (Eisenberg, l. c., p. 12).

[3] Eisenb., p. 16.

[4] Eisenb., p. 408 (quite distinct from Flugge's form: see above).

[5] Sternberg, Manual of Bacteriology, p. 720.

[6] Spaltpilze, p. 60; see also Cornil and Babes, Bactériologie, p. 142, and reference to a form mentioned by Pasteur.

[7] Zimmermann, l. c., p. 79; Maschek, Bakt. Unters. d. Leitmeritzer Trinkw., p. 60; Adametz, Die Bakterien d. Trink- u. Nutzwasser, No. 17.

form and his own as probably identical, and Lustig takes the same view.

It may be worth while to raise the question whether the *Sarcina*-form and the *Staphylococcus*-form of Micrococci are more than growth-forms of one and the same organism. If this turned out to be true, Schroeter's *Sarcina rosea* --and possibly Menge's *Sarcina* of red milk [1] is the same organism—would have to be examined in this connexion.

Several of my micro-cultures in broth-drops showed, as we have seen, that this *Micrococcus* forms evident *Sarcina*-like groups when young and growing slowly, but that the botryoidal (*Staphylococcus*-like) growth prevails later on when development is rapid.

It is perhaps not incorrect to say that the few known forms of *Sarcina* all come from sources (acid media, air, water, &c.) which may be regarded as poor pabula for such organisms. In any case there is nothing absurd in the suggestion, because it is known [2] that *Sarcina*-forms may so alter their habits on certain food-media that the cells become isolated by dissolution of the membranes and only single *Micrococci*, or (when dividing) *Diplococci*, are found, though the 'packet-form' can be obtained by another alteration of the food-medium.

I regard the case as not only interesting, but of some importance, for no one would have been able to infer the existence of the two conditions without actual culture in hanging-drops.

This form was easily revived on July 13 from an Agar culture of the preceding Aug. 14—i. e. eleven months and soon came up normal.

Its peculiar cherry red (cerise) colour and other characters were as before, and it was interesting to see how the differences between it and certain other red species—e.g. *B. prodigiosus*—were maintained.

[1] Centralbl. f. Bakt., VI, p. 596.
[2] E. g. Macé, l. c., p. 364.

IV. A Pseudo-bacillus [1]. (Plate XXI.)

This occurs as irregular and often curved rods 4 × 1 μ in water, motionless, often with spore-like darker spots in them, and breaking up into cocci. In old gelatine-cultures only the cocci are found, in chains or groups, or as diplococci and single cells, about 1 μ or a little less when stained. They stain by Gram's method.

No true endogenous spores have been found, though easily stained oval bodies occur in the rods as described.

In broth the motionless rods are often slightly curved, and measure 2–3 × 1–1·2 μ, and grow out to short filaments 10–12 μ and segmented. In some cases inflated involution forms occur, nearly 2 μ thick.

Plate-cultures at 12–15° C. show in four days as raised yellowish-white colonies, fairly quickly growing, and already coalescing. The submerged ones are very opaque, yellowish white, not zoned. Liquefaction begins in a week, as a slight sinking, but does not progress (Fig. 1).

After three months in culture, plates at 18–20° showed nothing to the unaided eye until the third day, but in forty-eight hours the ⅓ detected minute pale discs. On the third day just visible as white points, which under the ⅓ are greyish, hyaline, coarsely granular.

On the fifth day they look like raised drops of milk, 1 mm. diameter, domed, opaque, glistening yellowish white. Under ⅓ course, grey-yellowish, and opaque.

On the sixth day they are 1·5–2 mm., on the seventh 2–3 mm., opaque, cream-coloured, flattened domes. On the ninth day 3–4 mm., shining and like drops of cream. No trace of sinking, though some run together when in contact. The peculiar glistening appearance of the colonies is due to their wetness—as if sweating water on the surface.

Stab-cultures at 12–15°. In two days a raised dome-like button, porcellanous white, and slight yellowish dots in tunnel.

[1] Referred to as type of Group XVIII in Proc. R. S., Vol. lxi, p. 421.

In a week the colony above is a pure white, much raised, and shining like wet glazed porcelain. In a month it becomes cream-like and soft.

At 20° it grows equally well on acid and alkaline gelatine. In three days it is a very white raised button, 2–3 mm., with slightly confluent dots in tunnel. On the fourth day it is like porcelain, thick, glistening, raised. After about the sixth day it acquires concentric zones and a cream-colour, and looks as if turned (terraced) out of cream-coloured porcelain. No liquefaction, even in ten weeks.

Streak-culture at 20°. Cream-coloured, raised, glistening streak in forty-eight hours, and this grows fairly rapidly (Fig. 9). In a week it is a thick, glistening, creamy porcelain-like patch, broader below. No liquefaction in two months.

Agar at 30°. Forms a feeble streak, very thin, which makes no progress after forty-eight hours, but fades out as a transparent film. Invisible in eight days. At 35° also no growth in five days, whereas cultures at the same time at 23–25° formed a milk-like, broad, thin, shining, gummy or waxy streak with dense yellowish-white deposit all through the drainage (Fig. 10).

Potato at 22°. In twenty-four hours a wet spot, like dew. In three days this is a diffuse thin streak like milk and water. It thickens on the fourth day to a grey paste, and in a week is a not very extensive patch of cream-like, flesh-coloured paste (Fig. 8). On normal potato the growth is much more raised and distinctly flesh-coloured than on alkaline potato, perhaps because the potato acquires a pale violet hue showing it up. In ten days or so, both cultures are like rich buff or flesh-coloured cream.

At 34° the growth fails. A dew-like patch forms at first, but shows no advance in six days.

But on keeping the tube at lower temperatures, the characteristic flowing cream-like patch forms after some time.

Broth at 25° shows traces of turbidity in forty-eight hours, and a slight deposit in three days. On the fifth day a copious yellowish-white deposit. In a week, still turbid and a white

ring. In three weeks still turbid, white ring, and copious yellowish or buff deposit.

Milk at 25° shows no change in fourteen days. In three weeks it is just acid, but no apparent alteration.

Glucose at 25°. A slight white deposit in three days. In three weeks greasy flecks above and a fairly abundant yellowish-white deposit.

This form is not pathogenic to guinea-pigs, according to Professor Kanthack.

It was easily revived from an Agar-tube which had laid quiescent from May to June in the following year—i. e. thirteen months. It came up very pale and weak at first, but soon recovered all its normal characters as described. From the sum of the characters, including the results of microscopic cultures below, this form presents resemblances to *B. diphtheriae* which cannot be neglected, ***but it is not a Bacillus.***

When I came to make micro-cultures of this organism in hanging-drops of gelatine and of broth, some unexpected results were obtained of considerable interest and importance. The following examples will illustrate this:—

A gelatine drop-culture twelve hours old had a rodlet 4 × 1 μ at 8 a.m. (t. = 21° C.), which was fixed and observed under the Zeiss E as shown in Fig. 6 (*a–k*). At 9.30 the much longer and sharply bent rod was behaving very curiously for a Schizomycete, for it appeared to be ***putting out a branch at right angles*** from its lower segment (Fig. 6 *c*).

At 10.30 the much diluted gelatine was nearly fluid and an end-segment had broken off to the right and floated somewhat to the middle of the parent rod and there divided. The further course of the formation of the colony is visible in the drawings (*d–h*). At 4.40 p.m. a circular colony 24 μ in diameter had been formed (*i*): at 8 p.m. this was 32 μ in diameter (*j*). Next morning at 9 o'clock it measured 75 μ across (*k*) [1], and by noon it was 90 μ in diameter and quite typical.

[1] Sketched under a lower power.

Now it is pretty clear that apart from that curious lateral branch, there is very little to denote that this is not a typical Schizomycete, the segmentation of which is at first into rather long rods (10–12 μ) and then into shorter ones (about 3–4 μ). But there was no doubt that the branch *was* a true branch, and further examination in hanging-drops under the $\frac{1}{12}$ and $\frac{1}{18}$ immersion led to the proof that this organism is *not a true Schizomycete at all*, but an oidium-stage of an extremely minute fungus.

The following series (Fig. 5), traced under the $\frac{1}{12}$ in a broth-drop, will suffice to demonstrate this.

At 6.15 a.m. a rodlet (*a*) 3 × 1 μ was fixed, and at 8.40 a.m. it had grown out to a short curved filament (*b*) about 12 μ long: this was longer and distinctly segmented (*c*) at 9 a.m., and just before 10 o'clock (*d*) the longer segment was forming two branches, which had grown considerably by 11.10, and at 12.30 p.m. had crossed one over the other (*e* and *f*). The long segments now showed several septa, not easy to see but certainly visible with careful focussing. At 2 p.m. (*g*) the segments were breaking apart, after further growth *of the terminal ones*, i. e. the growth was *not intercalary*. At 7 p.m. (*h*) quite a large colony of separated segments, like rods, had formed, only part of which is figured. And next morning the still more broken up rod-like segments—some curved—had spore-like, brilliant oval bodies in them (*j*). These are of the nature of oidia or *chlamydospores*, in fact. These stain easily, with the ordinary alkaline methylene-blue, for instance.

As the figures (Figs. 4, 5 and 7) show, these stainable points appear before the final segmentation of the rods into coccus-like short joints - oidia – and then the membrane appears to thicken round them, converting them into spore-like *chlamydospores*.

Now, the point of special interest is that here we have an organism which, according to all its properties as tested by ordinary bacteriological methods, is a *Bacterium*. Its microscopic appearances, as shown in stained preparations, its behaviour in plate-cultures, and on and in all the usual media

employed by bacteriologists, all suggest its being a Schizomycete. Nothing but cultivation in hanging-drops could have demonstrated the fact that it is not a true Schizomycete at all, but an extremely minute Fungus—at least, I presume no one will dispute that its apical growth, acropetal mode of branching, and other morphological characters constitute more important tests than the bacteriological ones. Such forms are quite common among the Basidiomycetes[1].

In case any one should dispute this, however, it rests with him to construct a new definition of the Schizomycetes. Meanwhile, I emphasize the point—a point which I have insisted on elsewhere—that minuteness, staining reactions, rapid growth and the characters obtained in plate-cultures do not prove that an organism is a Schizomycete, and nothing but micro-cultures, difficult as they may be and are, can ever decide the point.

This point raises another matter of considerable interest, however, viz. that of the multiple origin of the group commonly known as Bacteria, by which I mean not only the Schizomycetes proper, but the totality of micro-organisms usually grouped with them.

Excellent evidence exists for the view that the true filamentous Bacilli (the Eu-bacilli or Endosporous Bacteria of De Bary) and the segmenting Bacteria which form no Endospores (De Bary's Arthrobacteria) must be regarded as having their origin from among the lower Algae, and it is customary to refer the former to groups like Oscillatoriae and the latter to forms like *Nostoc*. Whether the group to which *Cladothrix*, *Leptothrix*, and *Beggiatoa* are commonly referred can be joined to these is a debatable point.

For the various forms of *Sarcina* and *Micrococcus*, again, it is not difficult to find analogous forms among the lower Algae, e.g. Chroococcaceae and Palmellaceae, though it must not be forgotten that Micrococci are often merely the ultimate segments of anthrosporous filaments.

Without entering into the discussion as to alliances between

[1] See Brefeld, Unters. aus d. Gesammtgebiete der Mykologie, Heft 8.

various forms of Bacteria (in the wide sense) with Protozoa and Myxomycetes, and merely admitting that such alliance may well exist among the group, I would simply refer to a possible source of confusion which has become more and more probable since Brefeld has made us acquainted with the frequency of oidium-forms and chlamydospores among the Fungi [1], namely that these forms when very minute may easily be confounded with Schizomycetes. The only test is the acropetal mode of growth.

That minute yeast-forms are also liable to be mistaken for Micrococci is evident. I had recently in my laboratory a minute organism which grows in Canada-balsam, and am as yet unable to say with certainty whether it is a yeast-form or a Schizomycete.

Here then we have a good deal of matter for further research, for it is almost certain that minute organisms which will grow in gelatine and other media, and which stain by ordinary methods, are continually being descrjbed as Schizomycetes without the application of the only test which really decides the question.

I am strongly inclined to the opinion that we shall have to revise our views as to the divisions of the accepted Schizomycetes very much before long. For instance, Fischer's recent work on the cilia of Bacteria [2] seems to raise the question whether we must not assume a different origin for the ciliated forms of 'bacilli' and for the non-ciliated ones; and, in view of Ali-Cohen's discovery of a ciliated 'Micrococcus' [3] (*M. agilis*), the same applies to the Micrococci.

In any case it is difficult to avoid the conclusion that the organisms grouped under the common denomination of Bacteria (in the wide sense, but including obvious Fungi) are a heterogeneous collection of organisms with very different alliances, some of which have been indicated [4].

[1] Brefeld, l. c. [2] Unters. üb. d. Bau d. Cyanophyceen und Bakterien.

[3] Centralbl f. Bakt., Band vi, p. 33.

[4] Migula, System d. Bakterien, 1897, and Fischer, Vorlesungen uber Bakterien, 1897, have recently proposed extensive revisions of the classification, and have raised similar questions, but not quite the same points as I have here suggested.

Another point of importance, however, concerns those endosporous bacilli which are never motile, e.g. *B. Anthracis*, and those which have cilia, e. g. *B. subtilis*. I believe no one has suggested that the former may have had a totally different origin from the latter and that both may have been derived from ancestors other than Cyanophyceae; but it seems not impossible that minute reduced forms of Zygnemaceae and allied Conjugatae may have given rise to the non-motile bacilli. In such an event the endospores are probably homologues of azygospores[1], the intercalary growth, division, shapes of cells, and even the tendency to gelatinization of the cell-walls remaining the same.

Indeed we may go further. Many Ulothricaceae would serve as prototypes of ciliated bacilli if they lost their chlorophyll and became reduced. It is not impossible that we may have to abandon the Cyanophyceae as probable ancestors of endosporous forms altogether, for none of the Oscillarieae develop ciliated cells, while many Chlorophyceae have intercalary growth and gelatinous walls.

Even the curious pedicellate bacilli, which form one-sided growths or stalks of gelatinous consistence, such as my *B. vermiforme*[2] and the *B. pediculatum* of Koch and Hosaeus[3], are not without possible parallels among Chlorophyceae, e. g. Naegeli's *Oocardium*[4] and other Tetrasporeae.

Moreover, it would seem probable that some of the Chlamydobacteriaceae have had a totally different origin from any of the other Schizomycetes, as is especially evident when forms like *Phragmidothrix* are compared with *Bangia* and its allies.

The development of endospores has undoubted analogies with the formation of cysts in certain Flagellatae—e. g. *Chromulina* and *Monas*—as Migula has pointed out[5], and there are several other cases.

[1] Klebs, Die Bedingungen d. Fortpflanzung einiger Algen, &c., p. 255.
[2] Phil. Trans., Vol. clxxxiii, 1892, p. 149.
[3] Lafar, Technische Mykologie, p. 247.
[4] Pflanzenfamilien, 1. Th., 2. Abth., p. 51, Fig. 33.
[5] Pflanzenfamilien, 1. Th., 1. Abth. a., p. 11.

The Schizo-saccharomycetes, again, form a group which suggest obvious relationships to the yeasts, while Thaxter's Myxobacteriaceae point to alliances with the Myxomycetes.

All things considered, therefore, I think we should be prepared to accept that the morphological relationships of the minute organisms grouped together as Schizomycetes are neither few nor simple, and that their phylogeny is probably not even comparable with a complex tree-form, but is multiple in origin.

EXPLANATION OF FIGURES IN PLATES XX AND XXI.

Illustrating Professor Marshall Ward's paper on Thames Bacteria.

PLATE XX.

I. Short Colourless Bacterium.

Fig. 1. Rodlets and cocci *a* actively growing on gelatine at ordinary temperature; *b* on gelatine at 20° C.; *c* form from an old Agar-culture.

Fig. 2. Gelatine plate-colonies at 20° C. *a* on the third day, *b* on the sixth day after making plates.

Fig. 3. Culture from single rodlet. At 3 p.m. a rodlet (one of two) $= 2 \times 1\ \mu$ was fixed in gelatine at 20° C. *a*, at 10 p.m. this had divided *b*, and at 11 a.m. next morning it had formed the colony *c*: see p. 288.

Fig. 4. Stab-gelatine, one month at 20° C.

Fig. 5. Streak-gelatine, one week at 20° C.

Fig. 6. Agar-culture, four days at 20° C. The layer in *a* consists of coalescent colonies shown slightly magnified in *b*.

II. Capsuled Coccus or Bacterium.

Fig. 1. Plate-colonies at ordinary temperatures, a week old. *a* an emerged colony under $\frac{1}{3}$ showing the characteristic streakings; *b* a submerged colony natural size, and *c* the same under $\frac{1}{3}$ showing the gland-like appearance; *d* a portion of *a* under E/4, showing the embedded zoogloea-masses.

Fig. 2. Three submerged colonies, two days at 20° C.

Fig. 3. A piece of Agar-culture, showing embedded zoogloeas: *a* under $\frac{1}{3}$; *b* under $\frac{1}{12}$ imm. in water; and *c* the same stained with methylene blue, showing the 'capsule' round the masses embedded in gelatinous matrix. The capsuled masses average 4–6 μ to larger and smaller: the organisms, $2 \times 1\ \mu$ to $1 \times 1\ \mu$.

Fig. 4. Stained specimen after passage through animal; preparation from plastic lymph $\frac{1}{12}$ imm. Capsules hardly visible; cocci $1 \times 0{\cdot}75$ to $1 \times 0{\cdot}9\ \mu$.

Fig. 5. Similar from peritoneal fluid: the 'capsules' visible. Cocci about 0·75 to 1·0 μ.

Fig. 6. Similar preparation from Agar-culture stained by Gram's method. 'Capsuled' masses 6–10 μ; rodlets $1 \times 0{\cdot}6$ to cocci about 0·6 to 0·75 μ.

Fig. 7. Colonies after one year's rest, seven days' plate at 18–20° C. Nat. size.

Fig. 8. A ten days' stab-culture showing commencement of the liquefaction.

Fig. 9. A nine days' streak-gelatine-culture.

Fig. 10. An Agar-culture after revival. Five days at 35° C.

Fig. 11. A potato-culture (revived), three days at 25° C.

Fig. 12. *a*, a rodlet 2·5 × 1 μ sown at 3 p.m. in gelatine-drop at 25° C; *b*, the same, divided into four rodlets at 10 p.m. $\frac{1}{12}$ imm.

Fig. 13. A similar culture showing the oscillating movements in the partially liquefied gelatine. In *a* the pair of rodlets together end to end; *b* they have flown apart; *c* come together again; *d* again apart. This oscillating movement may concern both or only one rodlet at a time.

Fig. 14. Similar culture. *a* rodlet fixed at 10.25 p.m.; *b* the same dividing at 11 p.m.; and *c* oscillating apart at 11.5 p.m. At 7 next morning the colony *d*, 25 μ in average diameter, had been formed.

Fig. 15. *a*, a rodlet fixed at 4 p.m. This remained at 15° C. through the night: at 8.15 a.m. next day it had given rise to 8–10 bacilli *b* forming a minute colony 3 μ diam. The temperature then slowly rose to 25° by 3 p.m., when the colony *c* had resulted, about 15 μ diam. At 8.30 p.m. the rodlets were actively swarming, but came to rest during the night.

Fig. 16. A series showing division &c. of rodlets in gelatine. *a* at 10 a.m.; *b* at 10 20; *c* at 10.35; *d* at 11.40; *e* at 12.20; *f* at 12.45. t. = rising from 21·5° to 23·5° (see p. 301). $\frac{1}{12}$ immersion.

PLATE XXI.

III. Rose-pink Micrococcus.

Fig. 1. Plate-colonies at 15–20° C. for ten days: nat. size.

Fig. 2. The same in a month: nat. size.

Fig. 3. The same, six weeks, under $\frac{1}{2}$.

Fig. 4. Stab-culture, five days at 20° C.

Fig. 5. Agar-culture, three weeks at 25° C.

Fig. 6. Potato-culture, fourteen days at 20° C.

Fig. 7. Groups of cocci under $\frac{1}{12}$: *a* fresh; *b* stained.

Fig. 8. Broth-drop culture under $\frac{1}{12}$, showing Sarcina-form: *a* at 11.50 a.m.; *b* at 2.30 p.m.; *c* at 4 p.m.; *d* at 9.50 p.m.

Fig. 9. Characteristic groups from a strong culture at 20° C. on third day under $\frac{1}{2}$, showing the glandular botryoidal masses.

Fig. 10. Gelatine-drop culture under $\frac{1}{25}$ imm. *a* at 5.55 p.m.; *b* = 8.5 p.m.; *c* = 11.40 p.m.; *d* = 9.10 a.m. next day; *e* = 12 noon; *f* = 2.10 p.m.; *g* = 4 p.m.; *h* = 9 p.m. Temperatures fell from 20° to 16° C., then rose to 22° C.

IV. A Pseudo-Bacillus.

Fig. 1. Plate-colonies: *a* on fourth day at 15–18°, under $\frac{1}{2}$; *b* after fourteen days at 20° C. Nat. size.

Fig. 2. Plate-colonies at 20° after seven days. Nat. size.

Fig. 3. Rodlets from an old gelatine-culture, $\frac{1}{12}$ imm.

Fig. 4. Rods stained with methyl-blue, $\frac{1}{25}$ imm., showing spore-like bodies.

Fig. 5. A rod *a* in broth-drop at 17·5–19° C., under $\frac{1}{12}$ imm., showing

growth. *a* at 6.15 a.m.; *b* at 8.40: *c* at 9.0; *d* at 10; *e* at 11.10; *f* at 12.30 noon; *g* at 2 p.m.; *h* at 7 p.m.; *j* at 11 a.m. next day.

Fig. 6. A series at 21–23° C. in gelatine-drop under E/4. *a* at 8 a.m.; *b* at 8.50; *c* at 9.30; *d* at 10.30; *e* at 11.25; *f* at 12.50 p.m.; *g* at 2.15; *h* at 3.50; *i* at 4.40; *j* at 8 p.m.; *k* at 9 a.m. next day ($\frac{1}{3}$).

Fig. 7. Preparations in broth *a*, *b*, and *c*, and stained with methylene blue *a* under $\frac{1}{10}$ imm.

Fig. 8. A potato-culture, three days at 20° C.

Fig. 9. Gelatine-streak, three days at 20° C.

Fig. 10. Agar-culture, a week at 25° C.

Fig. 1.

Fig. 2.

Fig. 4.

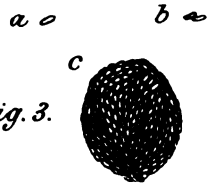


Fig. 3.

Fig. 5.

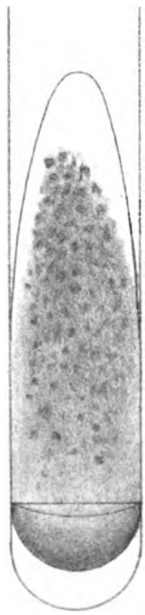


Fig. 6.

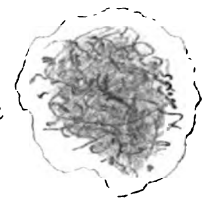


Fig. 1.

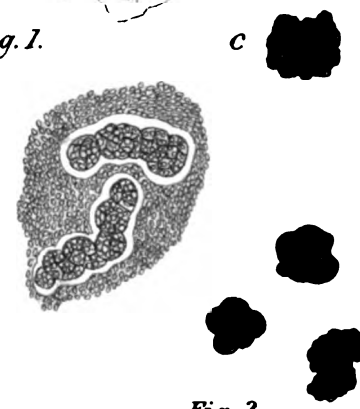


Fig. 2.

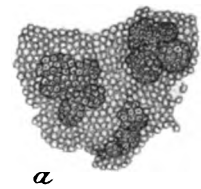


Fig. 3.

Fig. 4.

I, Short colourless Bacterium.

H M W. del

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11

a

Fig. 12.

b

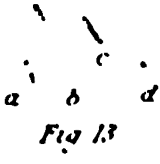


Fig. 13

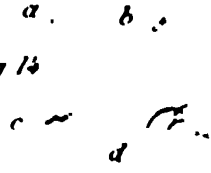


Fig. 14

Fig. 15

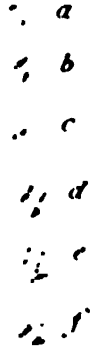


Fig. 16

[illegible] Capsuled [illegible] of [illegible]

Fig. 1.

Fig. 2.

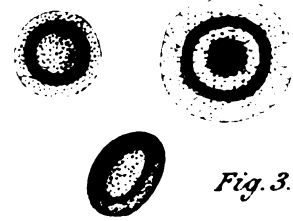


Fig. 3.

Fig. 4.

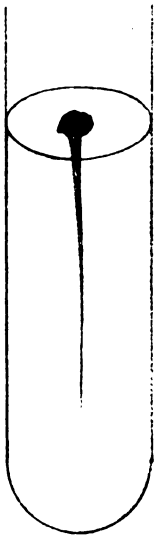


Fig. 5.

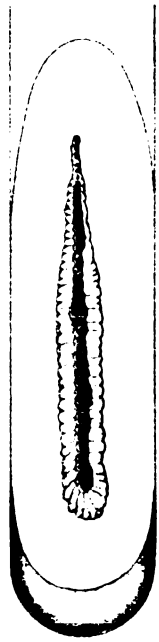
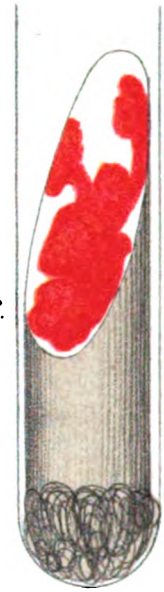


Fig. 6.



a

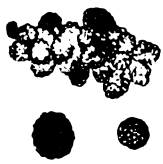
Fig. 7.

b

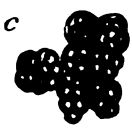
a

Fig. 8.

Fig. 9.



d



c

b

a

b

c

d

Fig. 10.

h

e

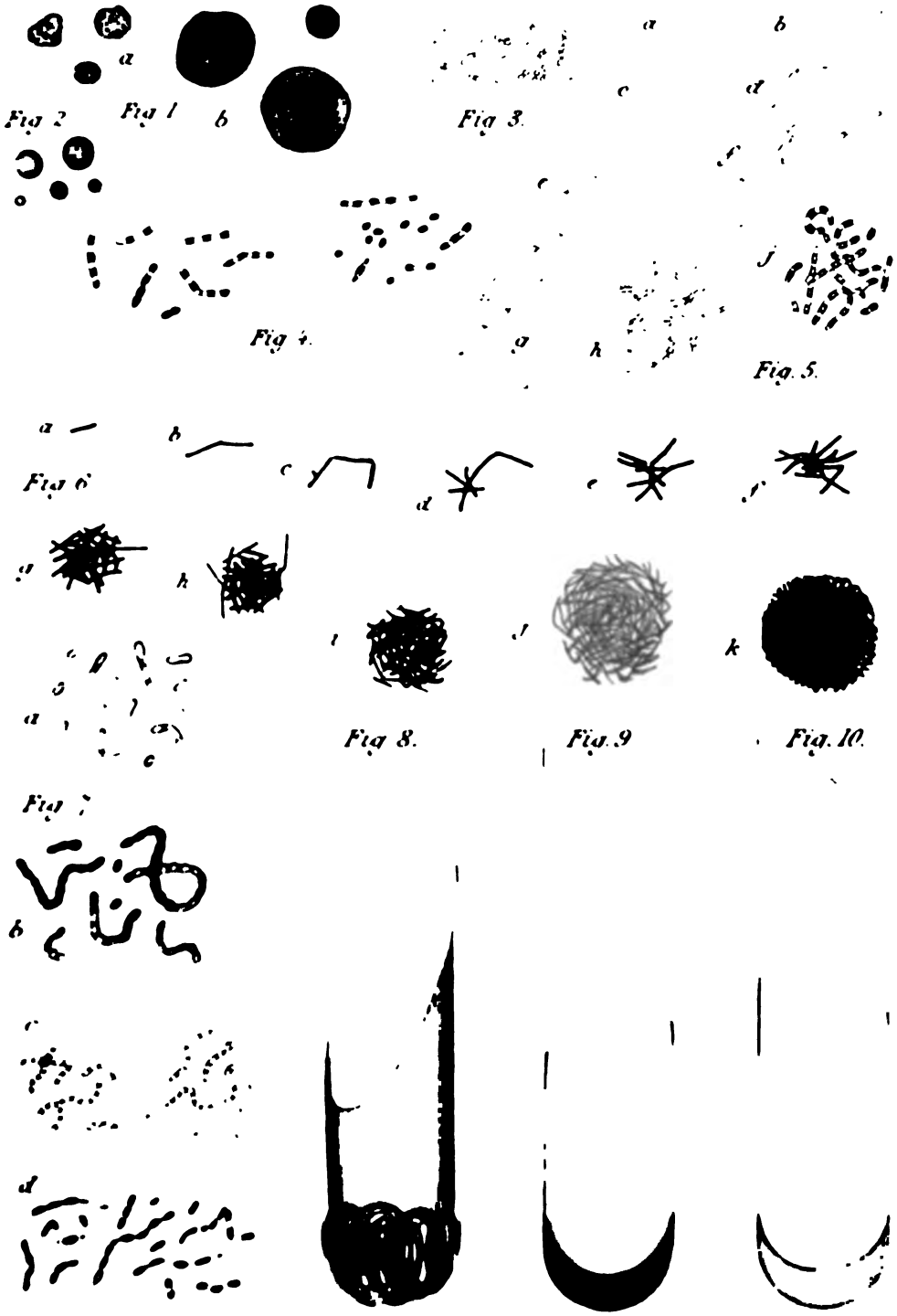
f

g

H M W del

III, Rose-pink Micrococcus.

WARD. – THAMES BACTERIA.



IV. A pseudo Bacillus

On the Roots of Bignonia.

BY

T. G. HILL,

National Scholar in Botany, Royal College of Science, London.

With Plate XXII.

HAVING had the opportunity of investigating the root-structure of a few species of *Bignonia*, it seemed desirable, as there exist some doubts as to whether the roots of these plants follow the anomalous structure of the stem, to put the results obtained on record.

As far back as 1850 Crüger[1] drew attention to the curious tuberous growths found on the roots of *Bignonia Unguis*, and he also stated that the same anomaly occurred in the roots as in the stem. This was subsequently denied by later investigators. Thus Bureau[2] says, 'M. Crüger . . . Il a suivi, dit-il, ce développement sur le *Bignonia Unguis*. J'ai pu examiner une racine de cette même espèce, et je dois dire que mes observations ne s'accordent point avec celles de M. Crüger.' Further on in his monograph he states: 'On ne voit point le tissu ligneux s'arrêter dans son développement sur quatre points opposés en croix, et le liber remplir les vides

[1] H. Cruger, Bot. Ztg., Vol. viii, pp. 109-110.

[2] Bureau, Monogr. des Bignoniacées, 1864, p. 149.

[Annals of Botany, Vol. XII. No. XLVII. September 1898.]

ainsi formés: mais il y a néanmoins une pénétration de l'écorce dans le bois.' It may, however, be stated that Bureau remarks that he had some doubts as to whether his plant was really *Bignonia Unguis* and not *Stigmaphyllon*; he decided it was the former, on account of the character of the sieve-tubes. Judging from further remarks which he makes on the root-structure of this plant, it is very probable that his material came from a wrongly named specimen. De Bary[1] states that he was unable to find the characteristic stem-structures in the roots of *Bignonia capreolata.*

Van Tieghem[2] states at the end of his description of the stems of these plants: 'Les racines de ces mêmes Bignoniacées ne paraissent pas présenter ces anomalies.'

Before passing on to the root-structure of these plants it will, perhaps, be well to briefly describe the structure which obtains in the stem for the sake of comparison.

Secondary thickening begins quite normally, as in other dicotyledonous woody plants. Sooner or later the development of secondary wood slackens very much at four points arranged cross-wise; and as the secondary growth of wood at the intermediate portions of the circumference continues at the same rate as before, it follows that four depressions are left dipping down into the secondary wood: but inasmuch as the formation of phloem at these four points is increased in inverse proportion to the decreased formation of wood, it is obvious that the depressions are quite filled up with phloem as far as the outer limits of the bast, so that the general external form of the stem remains similar to that of an ordinary plant. These four phloem-wedges are very characteristic of many Bignonias. The cambium at the bottom of each depression still slowly forms xylem-elements, and in the cortex of the stem numerous sclerenchymatous masses occur.

An essentially similar structure obtains in the root. In the ordinary root four phloem-wedges are found, and their development is identical with those of the stem.

[1] De Bary, Comp. Anat., Eng. ed., p. 573.
[2] Van Tieghem, Traité de Botanique, p. 823.

We will now turn to a more detailed description of the root-structure in various species.

Bignonia Unguis. In this species the anomalous structure of the stem is undoubtedly present in the root. The structure of the normal root without any sunken phloem is illustrated by Fig. 1, the protoxylem and the medullary rays are well marked and quite typical. With this illustration might be compared Fig. 2, which is a somewhat diagrammatic representation of a transverse section of an older part of the root figured in Fig. 1. It will be observed that the formation of sunken phloem has gone on to some extent.

The first indication of the sunken phloem makes its appearance in quite small roots from ·8 to 1 mm. in diameter, with five to ten cells in the radial rows of secondary wood. Such a stage is indicated in Fig. 3, which illustrates a transverse section of a young root. It will be seen that that protoxylem (*pxy*) is well marked, and is typically that of a root both as regards structure and position; secondary growth of wood has not gone on to any great extent; the phloem exhibits very fine sieve-tubes and companion-cells; and finally the cortical sclerenchyma is made up of fibres lignified to a very great extent, so that the lumina are quite small. In these fibres very fine simple pits were to be seen in the preparation from which this figure was taken.

On the other hand, in some roots with about 26 cells in the radial rows of secondary wood the process has not gone much farther; thus it seems that the development of the sunken phloem does not originate at the same time in equally developed roots. In roots of about 1·3 mm. diameter the development has proceeded until, with a diameter of 1·76 mm., the stage has reached that figured in Fig. 4. In some roots of a smaller diameter than the last, viz. 1·5 mm., the development has gone on to a greater extent, the sunken phloem being ·28 to ·3 mm. in depth (measured from the outer limit of the wood). Such a stage is indicated in Fig. 5.

Crüger[1] states that the anomaly is not so regular as in

[1] Loc. cit.

the stem. I have been unable to verify this. Most of my preparations show a very regular structure, e.g. those of the stage indicated in Fig. 5, and only in one case have indications of six phloem-wedges been seen.

The roots of *Bignonia Unguis* have a further interest in the fact that, at intervals, they swell out into tuberous growths resembling those occurring in the roots of certain species of *Asparagus*; they may attain a diameter of about 1 cm. and a length of about 1½ cm.

In a transverse section it is seen that there are many points of similarity between the tuberous and the ordinary roots. For instance, there is a well-developed periderm, and in the cortex there are numerous masses of sclerenchymatous elements. In the tuberous roots, however, they are arranged more regularly in concentric rings, and the masses also grow smaller towards the periphery; the sieve-tubes and companion-cells are well marked; and finally there are well-marked protoxylem-groups.

The chief points of dissimilarity between the tuberous and other parts of the root lie in the great development in the former of parenchyma in the cortex and, to a lesser extent, in the pith; and also in the breaking up of the xylem into separate masses, often by a certain amount of dilatation parenchyma.

There can be no question as to the structures described above belonging to the roots. In the first instance the material was carefully examined for any evidence of stem-nature in the shape of buds, &c., but without any success; then again the tuberous growths on the root are characteristic of the genus, the externally placed protoxylem is typically that of the root, and finally no phloem was found opposite the groups of protoxylem.

Bignonia venusta closely corresponds with *Bigonia Unguis* in the possession of sunken phloem in the roots. The first indications of the anomaly were found in roots of about ·97 mm. in diameter: in other roots of about 1 mm. diameter the phloem-wedges were about six cells in depth, while in

roots of 1·4 mm. in diameter the depth was about twice as great.

Bignonia capreolata is the only other species which has been examined; its roots do not show the anomalous structure of the stem; and although my material reached a diameter of 3 mm. and the outline of the phloem was distinctly waved, still there were no indications of the formation of sunken phloem.

The general structure of the root resembles that of *Bignonia Unguis*, the chief differences being the great amount of cortical tissue, and the less abundant cortical sclerenchyma not grouped together in masses, but in many groups with a few fibres each, in this species.

EXPLANATION OF FIGURES IN PLATE XXII.

Illustrating Mr. Hill's paper on *Bignonia*.

All the Figures refer to *Bignonia Unguis*.

Abbreviations: *camb.*, cambium; *c. c.*, companion-cells; *co.*, cortex; *cr. ph.*, crushed phloem; *m. r.*, medullary ray; *ph.*, phloem; *pxy.*, protoxylem; *p. w.*, wedge of sunken phloem; *scl.*, sclerenchyma; *xy.*, xylem.

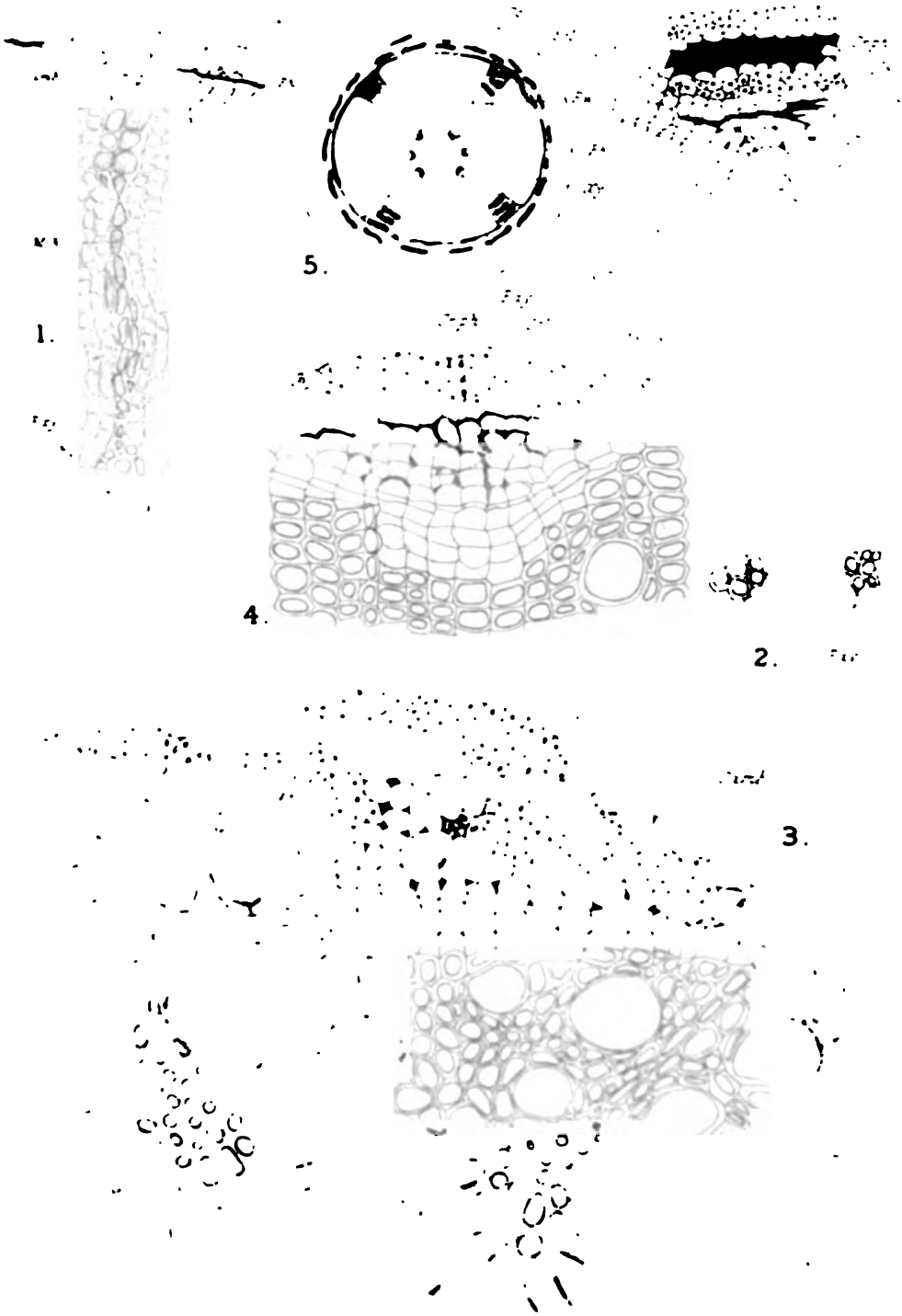
Fig. 1. Transverse section of a root.

Fig. 2. Transverse section of an older part of the same root as the last (somewhat diagrammatic).

Fig. 3. Similar section from another root.

Fig. 4. Transverse section of an older root showing an early stage in the development of the sunken phloem.

Fig. 5. Diagram of a transverse section of a root showing the sunken phloem well developed.



HILL ROOT OF BIGNONIA

Cupressinoxylon vectense; a fossil Conifer from the Lower Greensand of Shanklin, in the Isle of Wight.

BY

C. A. BARBER, M.A., F.L.S.,

Lecturer in Botany in the Royal Indian Engineering College, Cooper's Hill.

With Plates XXIII and XXIV.

INTRODUCTION.

IN the study of the anatomy of plants few subjects have received such minute attention as the structure of Coniferous wood. The simple and unique character of the elements composing such wood has rendered it peculiarly suitable for examining the growth of timber, the effect of seasonal variations and the formation of annual rings, the ascent of water, and other life-problems. But perhaps a greater inducement, at any rate among the older writers, was found in the useful and ornamental character of many of the fossil woods—coal, lignite, agate, opal, amber—which led to the formation of collections of these substances and their examination by the curious. It is hardly necessary to point out that the bulk of these woods belonged to the Coniferous type, this being due to their much wider distribution

[Annals of Botany, Vol. XII. No. XLVII. September, 1898.]

in former periods than at present, and the comparatively late appearance of the Dicotyledons in geological time.

Unfortunately the conditions required for the preservation of the minute anatomy of pieces of stems and roots of fossil plants differ widely from those under which the leaf- and bark- impressions have been handed down to us, and the two are therefore hardly ever found together[1]. This is not entirely due to the phenomenon of leaf-fall. The imprinting of the delicate venation of decaying leaves is largely a mechanical process, and pictures to the mind still lagoons and slowly-flowing streams; whereas the fragments of wood frequently bear the marks of much tossing to and fro before they were subjected to the chemical replacement of their cell-walls by silica, lime, or iron pyrites.

It is comparatively rare that the bark or cortex is left in fossils, and it thus happens that the sole guide to their systematic position is to be found in the minute structure of the wood. The uniformity of the elements in Coniferous wood has already been noted, and the problem here presented is seen to be of no common difficulty. Some of the best workers on the anatomy of plants have devoted their energies to its elucidation. Unger, Schleiden, Schacht, Mohl, Göppert, T. Hartig, Mercklin, Cramer, Kraus, Stenzel, Schenk, Conwentz, Felix, and many others, have produced a mass of monographs and treatises, almost all of them originating in an attempt to describe fossil woods, the necessity soon arising of extending their researches to an exhaustive study of recent Conifers.

In spite of these laborious investigations, the results have frequently been anything but satisfactory. Much labour was wasted by the earlier writers in attempting to unite each fossil wood with leaf or fruit remains occurring in strata of the same age. It was however soon demonstrated that classifications founded upon the structure of the wood could not be made to agree with those recognizing the natural

[1] Schenk, in Zittel, Palaeophytologie, p. 873, mentions *Sequoia Coutlsiae* among Coniferae. See also *Elate austriaca* in Unger, Chloris Protogaea, 1847.

affinity of plants. The independence of these systems was first insisted upon by Göppert, and to him therefore we owe our first real advance in the subject[1].

Göppert instituted certain classes or types of wood which he termed 'genera,' but which, so far from being synonymous with the genera of systematists, frequently united members of the most widely separated groups.

Göppert's genera, emended by Kraus[2], form the basis of all recent classifications of existing and fossil Coniferous woods. These may be arranged according to the following types :—

1. **Araucaria** Type. *Araucarioxylon.* Bordered pits small, touching, usually mutually compressed, several rows in each tracheide, the pits in adjacent rows placed alternately.

2. **Cupressus** and **Abies** Type. *Cupressinoxylon* and *Cedroxylon.* Bordered pits separate, in one row, or, if in more than one, the pits of different rows opposite one another, resin-canals absent, but strands of wood-parenchyma containing resin. In *Cupressinoxylon* the resin-parenchyma is abundant, in *Cedroxylon* scarce or absent.

3. **Pinus** Type. *Pityoxylon.* Bordered pits as in 2, resin-ducts with sheaths of wood-parenchyma among the tracheides and in the medullary rays, no separate wood-parenchyma strands present[3].

4. **Taxus** Type. *Taxoxylon.* Bordered pits and wood-parenchyma as in 2, no resin-ducts, tracheides with well-marked spiral thickenings.

Of these four types the first two are far more frequent in the fossil condition, the Araucarian with its allies extending from the Devonian to the present time, while *Cupressinoxylon* and *Cedroxylon* are more characteristic of the Secondary and Tertiary periods.

[1] Göppert, De Coniferarum structura anatomica, Breslau, 1841.

[2] Goppert, Monographie der fossilen Coniferen, Leiden, 1850; Kraus, Mikroscopische Untersuchungen uber den Bau lebender und vorweltlicher Nadelholzer, Würzburger Naturwissenschaftliche Zeitschrift, v, 1864.

[3] Schrocter, Untersuchungen uber fossile Holzer aus d. arct. Zone, Fl. fossil. arct., Oswald Heer, vi, Zurich, 1880.

It is doubtful how far it is possible to separate the last two forms of wood. Strasburger states that resin-parenchyma is to be found in all Coniferous wood except that of *Taxus*[1]. Beust asserts that the frequency of resin-parenchyma cannot be used in classification, the only division possible being into those with and those without it[2]. Certainly its absence from a few sections of a fossil would not demonstrate its typical absence from the species. Thus Felix, examining a specimen described by Schenk as *Cedroxylon*, because he found no parenchyma, was compelled to alter its name to *Cupressinoxylon*[3].

It becomes very difficult to draw a sharp line between the two types because of the many transitional cases. In young branches of *Cedrus Deodara* and *Cryptomeria japonica* examined by myself, the number of wood-parenchyma-cells per sq. mm. of transverse section was greater in *Cedrus* than in *Cryptomeria*, being fairly abundant in both. Yet *Cedrus* is included in the *Cedroxylon* type and *Cryptomeria* is a typical *Cupressinoxylon*.

Leaving out of consideration the collection of families included under *Araucarioxylon*, it is significant that *Cupressinoxylon* includes, among living forms, Cupressaceae, Taxodiaceae, *Podocarpus*, *Dacrydium*, *Saxogothaea*, *Phyllocladus*, *Ginkgo*, *Abies Webbiana*. The 'genus' therefore comprises the Cupressaceae in general as well as members of the Abietaceae, Taxodiaceae, and Taxoideae. Among Abietaceae are to be found genera or species belonging to *Cupressinoxylon* and *Pityoxylon*, and among the Taxoideae are included *Taxoxylon* and *Cupressinoxylon*.

These facts make it sufficiently clear that the character of the wood alone is of little taxonomic value. At the same time it cannot be altogether neglected. The section *Pityoxylon* offers characters distinct enough for determining

[1] Strasburger, Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen, Histologische Beiträge, iii, 1891.

[2] Beust, Untersuchungen über fossile Hölzer aus Grönland, Allgem. Schweiz. Gesellschaft, neue Denkschrift, xxix, 1884.

[3] Felix, Studien über fossile Hölzer, p. 29, Inaug. Diss., Leipzig, 1882.

the principal groups of *Pinus* and a certain number of species [1]. Under *Cupressinoxylon*, the genera *Sequoia*, *Phyllocladus*, *Ginkgo*, *Taxodium* and *Glyptostrobus* are said by different authors to possess characters by which they may be separated from all others [2]. A more careful study of the structure of gymnospermous wood might have placed this group between the Angiosperms and the Cryptogams instead of with the Dicotyledons as was done at first [3], just as Mercklin separated *Sequoia* from the Taxaceae on account of its wood-structure at a time when it was regarded as a member of that group [4], and as Kraus detached *Dammara* from *Cunninghamia* [5].

In spite of the fact that the woods grouped under *Cupressinoxylon* are drawn from such widely different groups of Coniferae, there is a truly remarkable similarity in their structure. Thus Kraus mentions forty-six species of recent Conifers, belonging to various groups, with woods of this type which he states are indistinguishable. This is the more to be regretted because the bulk of fossil Coniferous woods from the Jurassic to the Tertiary periods, and especially the enormous numbers found in the Brown Coal, belong to *Cupressinoxylon*.

The earlier workers do not appear to have appreciated these facts, and their diagnoses were lamentably meagre. They do not, moreover, appear to have had any adequate knowledge of the process of petrifaction, nor were they aware of the differences existing as regards wood-structure between different parts of the same plant. Branches, roots, stems, portions altered by chemical action or undergoing decay, were accordingly described as separate species, so that Kraus, in his analysis of work previous to 1864, ventured the extraordinary assertion that all *Cupressinoxyla* described

[1] Schimper, Traité de Paléontologie végétale.

[2] Schmalhausen, Beitr. zur Tert. Fl. Südwest-Russlands, Pal. Abh., Dames und Kayser, i, 1884. See also Kraus, Schroeter, Schenk in Zittel, and Goppert, l. c.

[3] Knowlton, Fossil Woods and Lignite of the Potomac Formation, Bull. No. 56, U. S. Geological Survey, 1889.

[4] Mercklin, Palaeodendrologikon Rossicum, St. Petersburg, 1855.

[5] Kraus, l. c.

up to that date might, from their diagnoses, very well belong to different parts of one and the same tree[1].

Many of the characters formerly used in descriptions, such as the width of the annual rings, numbers and heights of medullary rays, sizes of tracheides, thickness of walls, &c., have now been demonstrated to differ as much in the same species as in different genera. To illustrate this fact, Kraus carefully examined a fossil stem with branch attached. He showed that these two parts differed so widely in the characters just enumerated that, following the lines of the old-time diagnoses, they would have to be put into well-separated 'species.' The detailed study which has led to these results has indeed characterized the period inaugurated by Kraus (1864). Sanio, Schroeter, Schmalhausen, Russow, Kny, Schulze, Wille and others, have supplemented their general comparative study of many forms by a minute, intensive examination of individual species. In the process the earlier descriptions have been thrown into confusion, but the path has been marked out along which any work of value in this field must be followed.

It is true that these writers have shaken, one by one, the pillars upon which the classification of Coniferous woods has been erected; their work has, in this sense, been mainly destructive. The characters of absolute importance have, however, been rigidly defined, new characters have been raised from relative to absolute value; and, what is perhaps of equal importance, the conditions have been determined under which the characters of relative value may be used in diagnoses. These conditions include a knowledge of the age and morphological character of the part as well as the mode of petrifaction. It is nevertheless only too apparent that the work of reconstruction has only commenced, and any description of fossil wood which is to be of use in the future must include an exhaustive analysis of all the characters, both absolute and relative, which have not been proved to be purely fanciful.

[1] Kraus, l. c.

In view of this formidable and discouraging literature—an abyss in which such a vast amount of useless labour has been sunk—the description of a new 'species' of *Cupressinoxylon* is not an enviable task. But, as will be seen, it is possible to determine fairly accurately the age and morphological character of the specimens selected for description in the present paper; and it becomes in this way permissible to submit them to the same exhaustive analysis as would be made in the study of a recent wood.

The pieces of fossil wood dealt with are taken from a series of silicified, rolled, and water-worn fragments, much perforated by teredo-like burrows, collected from the Lower Greensand of Shanklin in the Isle of Wight [1].

Some of these specimens are in a beautiful state of preservation, showing not only the bordered pits on the radial walls with great distinctness, but even their sections in tangential view with what appears to be the suspended torus. Since a pith is to be found in all of them, it is easy to determine their approximate age; and, while it cannot be definitely stated which are stems and which are branches, certain of them show in a marked manner the arrangement of cells in the annual rings which are characteristic of roots. It has, further, been possible to obtain in each specimen several sections in the transverse, tangential, and radial directions (thirty-two sections in all), and by this means some of the minor peculiarities of individual parts have, it is hoped, been ruled out.

[1] I am indebted to Mr. A. C. Seward, F.R.S., for the first and most beautifully preserved specimen, and to Dr. D. H. Scott, F.R.S, for the others. In both cases the specimens were accompanied by sections to which I have added a considerable number.

The slides dealt with in the present paper have been numbered A. C. S. 4, 5, 6; D. H. S. 338, 344-349; C. A. B. 1, 2, 3, 7, 8, 9, 11, 12, 13, 17, 18, 23, 24, 29, 30, 35, 37-42. These sections have been cut by Mr. F. Chapman.

As regards the expense of section-cutting, this has been defrayed out of a small Government grant from the Royal Society. By this means I have been enabled to obtain a far more complete series of sections than would have been the case otherwise.

Description of Specimens.

Specimens 1–2 inches in diameter, with distinct, central, or excentric pith. Rings of growth well marked, averaging 1–2 mm. wide, composite, each with 2–6 bands of narrow dark summer elements. From the arrangement of the cells in the rings the specimens are considered to be young branches and roots. Pith well preserved, diameter in branches ·9 mm., in roots ·3 mm. to ·4 mm., cells increasing in size towards the centre, 10 μ to 50 μ in diameter, copiously pitted, with large triangular intercellular spaces 5–15 μ across. Medullary sheath: in the roots the rows of tracheides pass directly into the cells of the pith, in the branches they terminate in small groups of cells irregularly arranged. Spring tracheides not differing much in branch and root, tangential width 12–25 μ, radial 17–22 μ; summer tracheides, in the branches, radial diameter 10–11 μ, averaging 4–6 rows, in the roots radial diameter 12 μ, averaging 2–4 rows. Bordered pits in a single row (rarely double in roots), free and rounded in branches, often touching and compressed in roots, outer diameter 7–14 μ, inner 3–5 μ. Tangential pits frequent, occurring in 2–7 rows of summer cells, outer diameter 5–7 μ, inner 2–3 μ. Medullary rays simple, usually one, occasionally two cells broad, 1–16 cells high, the average being 2–3. Cells of ray 15–20 μ high, 12–16 μ broad, radial length various, covering 2–6 tracheides. Proportion of medullary-ray-tissue to the rest of the wood about 1 : 30. Resin-tissue, consisting of isolated rows of parenchymatous cells, abundant, equally distributed. Length of cells various, tangential width 19–28 μ, radial 11–20 μ.

It has not been found possible to place this wood under any species of *Cupressinoxylon* already described. The peculiarity of the rings of growth is probably in itself sufficient to establish a new species. Besides this, however, the youth of the specimens and the detailed examination to which they have been subjected, leave few points of comparison with known fossils. It will be better to defer

these comparisons until older specimens presenting the same peculiarities have been examined.

From the frequent occurrence of this type of wood in the Lower Greensand of the Isle of Wight, I have decided to call it *Cupressinoxylon vectense.*

Annual Rings.

The rings of growth are so peculiar as to deserve an extended description. They are compound in all the specimens (Fig. 1). They are readily visible to the naked eye, and each is seen to consist of a broad, inner, clear, and a narrow, outer, denser portion. The former, under a low magnifying power, is seen to consist of wide and more or less thin walled 'spring' elements. The latter is, however, not homogeneous, but is composed of a varying number of dark lines in a lighter matrix. The dark lines are narrow bands of thick-walled, flattened cells, and the lighter parts between them consist of wider cells with thinner walls resembling the cells of the inner portion of the ring but not so large (Fig. 2).

Under the microscope the dark bands of each series strongly resemble the 'autumn,' 'late,' or better 'summer' wood of living trees; but they are seen to vary much in extent and thickness, frequently anastomosing with one another or fusing together at short distances along the circumference of the section. What is one thin band at one place breaks up to from two to five in another or increases greatly in thickness. Besides these regularly grouped bands of flattened cells, other less definite ones may be traced at various points in the wide zone of spring tracheides. A close examination of this part of the ring shows that there are frequent changes in the radial width of the elements and the thickness of their walls, sometimes constant for some distance or indeed all round the section, at other times limited to a few cells only.

In the majority of cases the narrow dark bands would be classed as 'false' or 'partial' rings. They show a gradual

transition from wide cells within to narrow thick-walled ones, the latter passing again gradually into wide thin-walled elements outside (Fig. 2). The outermost band in each series usually presents the character of a 'true,' 'normal' or 'sudden' ring, that is a sudden change from flattened summer-cells to wide succeeding spring-tracheides. This sudden change in the character of the elements is usually regarded as correlated with a profound change in the vegetative activity of the plant; and for this and other reasons the broad rings of growth described above have been treated in the present paper as rings in the ordinary sense of the term.

It is not to be supposed that these rings of necessity represent annual periods of growth, for there are sometimes deviations from the above arrangement which would render such an interpretation full of difficulty. Thus it occasionally happens that the outermost dark band of a series is gradual, and more frequently one or more of the inner bands have the character of sudden rings. The same band at different parts of the section will change from sudden to gradual several times over. Lastly, in a few instances, a sudden ring appears in the midst of the wide spring-elements, can be traced for a short distance, and then completely disappears (Fig. 4). Taking into consideration this extraordinary variability, it will be seen that we have no proper data regarding the rate of growth of the plant to which the specimens belonged, especially as transverse sections taken at short distances from one another show great differences in the arrangement and composition of the rings of growth. The adoption of the term 'annual rings' in the present paper is to be regarded as a matter of convenience in describing the other characters of the wood.

This is not the place to review the voluminous literature dealing with the effect of climate on the structure of wood [1].

[1] For references to the principal papers dealing with this subject see especially Büsgen, Bau und Leben unserer Waldbäume, Kap. vii, Jena, 1897; Nördlinger, Deutsche Forstbotanik, i, 1874; and Seward, Fossil Plants as Tests of Climate, C. v, London, 1892.

It is important rather to determine how frequently irregularities of the nature described occur in recent and fossil species. There appear to be no analogous cases among fossil woods already investigated. Conwentz mentions that in the amber-producing *Pinus succinifera* the rings are distinctly visible to the naked eye, but under a lens they are found each to consist of a series of narrow rings, and he states further that he remembers having seen cases of a similar nature in other fossils[1]. Fliche figures an anastomosis between two rings of *Cupressinoxylon infracretaceum*[2], and Seward notes the presence of partial rings in his recently described *Pinites Ruffordi*[3]. But besides such isolated examples there appears to be nothing resembling our specimens among described fossils.

It is well known that in Dicotyledons of warmer regions the ring-formation, so regular with us, is deficient or absent. *Michelia* and *Avicennia* may be selected among many as resembling our fossil in the sharp character of the stem-rings, which nevertheless anastomose with one another, and thereby render the counting exceedingly difficult.

When however we turn to recent Conifers, we meet with much less irregularity. It has been considered worth while, in books devoted to forest-botany, to record cases of partial or indistinct ring-formation in this group. The anomaly is seen to be widely extended. Of more immediate interest to us are such plants or parts of plants in which this peculiarity occurs, if not habitually, at any rate more frequently than elsewhere.

We are told that branches are more subject to irregularities in wood-formation than stems[4]. In roots again, according to Nördlinger, it is frequently impossible to count the rings

[1] Conwentz, Monogr. d. baltischen Bernsteinbäume, 1890, p. 32.

[2] Fliche, Note sur les Nodules et bois minéralisés trouvés à Saint-Parres-les-Vaudes (Aube) dans les grès verts infracrétacés, Mém. de la Soc. Acad. de l'Aube, lx, 1896.

[3] Seward, *Pinites Ruffordi* from the English Wealden Formation, Journal Linnean Society, Bot. xxxii, 417.

[4] Felix, Beitr. zur Kenntniss foss. conif. Hölzer, Engler, Botan. Jahrb. iii, 1880, p. 265.

of growth even in those plants which have rigidly-defined rings in their stems and branches[1]. As will be seen directly, our specimens probably include both branches and roots.

I have observed gradual rings in branches of *Abies Pinsapo*, *Cryptomeria japonica*, *Juniperus virginiana*, and especially *Juniperus communis*, although the rings are well separable and easy to follow. The sections of *Cupressus sempervirens* in Nördlinger's famous series, on the other hand, show a confused arrangement of gradual and normal rings frequently anastomosing with one another, and rendering the counting a matter of considerable difficulty. The resemblance between these sections and our fossil is very striking. In the other cases just mentioned the gradual rings may be regarded as exceptionable disturbances in the wood-formation; in *Cupressus sempervirens* the irregularity appears to be the usual state of affairs. Thus Hartig describes two branches of this Cypress, one fifteen to twenty years old, showing only three rings of growth, and one twenty-five years old with nine rings[2]. Through the kindness of Mr. Thomas Hanbury I have been able to examine the branches and roots of two trees of this species from his garden at La Mortola. A study of these specimens confirms the previous observations. There are the gradual and the sudden rings, the anastomosis of the narrow dark bands, and even a tendency to the formation of bundles of small rings, usually two together, alternating with clear zones, which forms so marked a character of our fossil specimens. Such irregularity as this cannot be referred to external conditions, and, as we have seen, the rings of this *Cupressus* do not of necessity correspond to definite periods of time. I have accordingly felt justified in including the irregularity in the formation of the annual rings among the distinguishing characters of the fossil under discussion.

We are sometimes able, from a study of the arrangement of the tracheides in a transverse section, to tell whether it

[1] Nördlinger, l. c.

[2] Hartig, Vollst. Naturgesch. d. förstl. Kulturpflanzen Deutschlands, p. 86.

is taken from root. stem or branch. In the rings of a Coniferous stem, according to Mohl[1], there is a gradual decrease in the radial diameter of the cells from the first tracheides of the ring to the last, accompanied by an increase in the thickness of the walls. In a root-ring, on the other hand, a number of layers of wide spring-elements are succeeded suddenly by a band of flattened thick-walled summer-cells which terminate the ring. This difference is explained in the following way. In a wide ring, whether in stem, branch or root, there are three distinct zones which differ as regards the form and size of their cells—an inner layer of wide square cells, a middle zone of polygonal, usually hexagonal cells, and an outer zone of typical summer-cells which are again more or less rectangular. Stems generally have wider rings than branches or roots, and all three layers are met with in them. In branches the inner layer is variable, and in thin rings is frequently absent. In roots on the contrary it is the middle layer which varies, and in thin rings it is usually wanting.

The thin rings which characterize smaller roots and branches are thus seen to be very different in appearance. In branches a set of hexagonal cells passes gradually into the thick, flat summer-cells; in roots wide, square tracheides are followed without transition by the flatter layer, which, in this case, is often reduced to only two or three rows of cells.

On applying these facts to our fossil sections we note that they can with little difficulty be divided into two sets (Figs. 2 and 3). In one of these, which I have called branch (1) and branch (2), the cells of the inner layer are polygonal and pass gradually outwards to from 2–12–20 summer cells. In the other specimens, while in places there are gradual transitions, many parts show a sudden passage from wide, frequently squared cells, to from 1–2–4–6 flat dark tracheides. These I have called root (1) and root (2). It seems therefore probable

[1] Mohl, Einige anat. und physiol. Bemerkungen über das Holz der Baumwurzel, Bot. Zeit. 1862.

that the specimens are smaller branches and roots, an assumption which is supported by a number of other characters, although a few difficulties are met with.

It should be pointed out in passing that this distinction between root- and stem-structure must be used with a certain amount of caution. If, as Mohl holds, it has its explanation in fundamental differences in the function of the two organs, it will be capable of wide application [1]. There are, however, instances of stems exhibiting the typical root-arrangement. Thus Strasburger, in describing the last-formed layers of an old moribund Larch, states that the stem-rings are small and consist of very wide spring-tracheides followed suddenly by narrow thick-walled summer-cells. The same arrangement was found in the wide rings of a perfectly healthy Larch forty-eight years old. It is indeed stated by Nördlinger to be a common feature of Larch-wood [2]. Lastly, Mohl expressly remarks on the small difference between the outer stem- and root-rings of the Larch. It cannot then be considered altogether safe to use this character alone in determining the root- or stem-nature of fossil woods. To give an example, *Cupressinoxylon distichum*, described by Mercklin [3], is now regarded as a root, and yet the rings (which are stated to be the outer rings of a very thick trunk) correspond exactly with those of Strasburger's old dying Larch stem [4].

In one of the specimens the rings are wavy, i. e. thrown into a series of irregular arches around the section (Fig. 1). This has been noted as an occasional occurrence in many recent Coniferae; and among fossils in *Cupressinoxylon nodosum* (root) by Göppert [5] and *C. erraticum Terebinum* and *C. Fritscheanum* by Mercklin [3]. The arches render the measurement of the elements difficult, since the cells are much smaller where two arches meet, as if these regions were subject to pressure. Possibly this is due to the nearness

[1] Mohl, l. c. [2] Strasburger, l. c., p. 24. [3] Mercklin, l. c.

[4] See also Kobbe, Ueber die fossilen Hölzer der Mecklenburger Braunkohle, Inaug. Diss., Rostock, 1887.

[5] Göppert, Monograph.

of small branches, as in the case of Göppert's *C. nodosum*. See also his figure of a Larch-stem with many small lateral branches and wavy rings [1].

The width of the rings of growth as defined above is somewhat variable. This character is not now regarded as of diagnostic value, since the rings will vary in width according to climate, age, soil, and the part of the plant.

As the same ring differs considerably in different parts of its course, the measurements have been taken in all directions where the structure has been undisturbed.

Branch (1). Average width of first ten rings 1·7 mm., varying from ·6 mm. to 2·8 mm.

Branch (2). Average width of first ten rings 1·45 mm., varying from ·7 mm. to 2·3 mm.

Root (1). Average width of first twelve rings 1·07 mm., varying from ·4 mm. to 2·1 mm.

Root (2). The rings near the pith are quite indistinct. Then follow five rings with an average width of 1·7 mm., varying from 1 mm. to 3·4 mm. After this five narrow, dark bands occur at distances of from ·2 mm. to ·8 mm.

Pith and Medullary Sheath.

The pith has received careful examination whenever the sections have admitted. It is obvious that from its study great assistance might be expected in determining the root- or shoot-nature of organs. This expectation was only partially realized. In the first place the usual absence of pith from pieces of fossil wood has caused it to receive small attention in the literature of the subject, and secondly the state of preservation of the cell-wall did not allow the markings to be clearly seen at the critical points. The diameter of the pith in branch (1) and branch (2) was ·9 mm.; in root (1) it was ·4 mm., and in root (2) ·3 mm. This is in agreement with Nördlinger's statement that, while in

[1] Göppert, l. c., Tab. i, Fig. 10.

branches and in primary roots the pith is distinct, that of lateral roots is point-like if present at all[1].

An examination of the 'medullary sheath or crown' points in the same direction. While branch (1) and branch (2) have well-marked groups of primary xylem where the tracheides lose the radial arrangement (Figs. 5 and 6), roots (1) and (2) show rows of tracheides passing directly to the borders of the pith (Fig. 7). A search for the primary xylems in longitudinal and oblique sections of these specimens has not been very successful; but in the tranverse section of root (1) there is a distinct appearance of tracheides with spiral thickenings in the pith opposite several of the primary medullary rays.

In the radial sections the appearances are not so convincing. In branch (1) the tracheides with bordered pits are easily seen to be in contact with the spiral ones, these latter adjoining the pith-cells. This is what one would expect in a section of a shoot. But there is every appearance that the same is the case in root (1). The state of preservation in root (2) and branch (2) prevents their sections from throwing any light on the question.

The number of primary medullary rays varies in the sections. It is not possible to determine them accurately, but they seem to be between 2 and 7.

The cells of the pith in transverse sections are largest in the centre, from whence they decrease outwards to the 'crown' of tracheides (Fig. 7). The diameter of the former may be taken as about 45 μ, while the outer medullary cells measure 10–20 μ. In longitudinal sections the inner cells are seen to be flattened transversely, so as to extend across the pith, while the cells near the tracheides are elongated in the same direction as the latter.

All the pith-cells are richly pitted. Where undisturbed they are circular in transverse section, and have well-marked triangular intercellular spaces 5–10–15 μ across.

[1] Nördlinger, Forstbotanik.

The Tracheides.

The general arrangement of tracheides, as seen in transverse section, is fairly regular and similar to that in most woods of this class. Radial rows of broad cells alternate with narrow ones wedged in between them. The former represent the tracheides cut across at their broadest place, the latter the chisel-shaped ends with richly pitted walls. While the cells of each radial row are nearly constant in tangential width, neighbouring cell-rows differ very much.

In order to measure the *tangential width* of the tracheides it is obviously impossible to isolate the cells and measure them individually at their broadest part, as has been done by Schulze for recent woods [1]. All that can be done is to obtain a general average of the tangential width by counting the number of cells in a measured distance [2]. This number will depend partly on the length of the tracheides; the shorter these are, the more frequent will be the rows of narrow ends.

It has been already pointed out that the cells vary in their size in wavy rings, being apparently much compressed where the arches join. It has therefore been found necessary to multiply observations in order to obtain a true average. The results appear to be in accordance with the general laws laid down by Mohl, Sanio, Schulze, Kraus, and Conwentz. The following figures give the measurements of from 100 to 250 tracheides in each ring in eight different sections, the number of observations depending upon the state of preservation. They have involved the counting of over 17,000 cells.

[1] Schulze, Ueber die Grössenverhältnisse der Holzzellen bei Laub- u. Nadelhölzern, Inaug. Diss., Halle, 1882.

[2] Sanio, Ueber die Grosse der Holzzellen bei d. gemein. Kiefer, Pringsh. Jahrb. viii, 1892, p. 403.

Tangential width of spring-tracheides in μ:—

Rings	1	2	3	4	5	6	7	8	9	10	11
Branch (1)	12·5	18·8	20·7	22·7	23·7	24·3	24·5	24	24·7		
Branch (2)	14	17·5	20	21	23·5	24	25·5				
Root (1)	12·7	16	20	23	23·7	25	24	25·2	24·5	25	24·5

In root (2), the inner rings not being well defined, the tracheides were measured at about equal distances from the pith, and in successive rings where these were apparent.

Root (2) | 10 | 16·5 | 21 | 24 | 26 | 26 | 22 | 24 | 22 μ.

We thus see that the tracheides in the first ring are very small; from the second to the sixth a rapid rise takes place, and from the sixth to the eleventh the tangential width is fairly constant.

The *radial width* of the tracheides has been calculated by measuring the first ten tracheides in each ring in ten as widely separated regions of the section as possible. The resulting average of 100 spring-tracheides is assumed to be a fair guide. The measurement is, however, rendered difficult by the indefinite limits of certain of the rings, and the consequent necessity of selecting suitable rows for observation. No figures could be obtained for the first few rings, and in root (2) it was found necessary to adopt another method.

Radial width of spring-tracheides in μ:—

Rings	1	2	3	4	5	6	7	8	9	10	11	12
Branch (1)			17·2	18·7	19·1	20·6	21·5	22·3				
Branch (2)				20·4	21	23·3	22·2					
Root (1)				19·2	19·6	20·9	20·3	21·6	22·5	22·1	20·5	20·2

In root (2) well-defined rings are absent near the pith. Besides this, where the rings are visible, it is found that the largest cells are in the middle of the ring, those at the commencement being considerably smaller. I have, accordingly, measured the ten tracheides at regular intervals from the centre or in the middle of each ring.

Root (2) | 10·5 | 14 | 20 | 25 | 26 | 32 | 29 | 26 | 33 | 26 | 26 | 26 | 28 μ.

The branches show the increase in radial diameter which was to be expected. The roots show first an increase in width, followed by a decrease in succeeding rings. Without laying much stress upon this latter peculiarity, because of the fewness of observations, it may be pointed out that the arrangement is in accordance with the results obtained by Sanio. He showed that while in branches the increase in size of the tracheides is maintained until a constant is reached, in roots these elements increase in radial diameter during the first few years, then decrease, later on again increasing until a constant is reached [1].

It is important to note that, according to Mohl and other observers, the elements of roots are larger than those of stems and branches. This does not hold good in the present case, a fact which throws some doubt upon the division of our specimens into roots and branches [2].

An examination of the summer-tracheides, on the other hand, tends to support this division. The summer-elements of roots are stated by Mohl to be fewer in number and less flattened than those of branches and stems [3], and this appears to be the case in our section. I have collected for comparison the most flattened elements of each ring, and have in each case tried to get as many in a radial group as possible (cf. Figs. 2 and 3).

[1] Sanio, l. c.

[2] Nordlinger, Forstbotanik, mentions cases where there is not much difference between the size of cells in stems and roots (Aspen, &c.).

[3] Mohl, l. c.

Measurements of summer-tracheides.

	No. of groups measured.	Average number of tracheides in a group.	Average radial diameter of tracheides.
Branch (1)	20	4·3	11·1 μ
Branch (2)	13	5·4	9·8 μ
Root (1)	31	3	12 μ
Root (2)	25	3	12·2 μ

The walls of the tracheides are frequently seen to be striated in longitudinal sections. This is especially the case where, from the appearance of the bordered pits and the presence of fungus hyphae, it is evident that decomposition had commenced before petrifaction. The striation in these cases is probably due to this cause. In tangential sections, however, the summer-wood is seen to be striated even in well-preserved parts where the spring-wood is not—a phenomenon not due to decay, but often met with in the wood of recent Conifers.

The thickness of the walls of the tracheides, a character which was once considered to be absolute, varies a good deal with the state of preservation of the part. An average of 80 measurements gives 7·2 μ, ranging from 4 μ to 14 μ.

Bordered Pits.

The bordered pits have received the utmost attention from students of wood structure. By means of these, Coniferous wood is easily separated from that of Angiosperms, and of the former the Araucarian type is cut off from the rest. There are also subordinate differences in the sizes of the bordered pits and the numbers of rows per cell in stems, branches and roots. Since in Coniferae they occur exclusively upon the radial walls, the study of radial sections has acquired great importance in diagnosis.

The bordered pits in our sections are arranged in one row—probably in great part due to the youth of the specimens. A comparison of the radial diameter of the tracheides and

that of the pits shows that there is barely room for two rows of pits in one cell. We should not therefore meet with a double row of pits unless two tracheides were placed exactly opposite one another. This arrangement of cells, as already mentioned, is more frequently met with in the root-specimens, and we accordingly find one or two instances in the root-sections of two bordered pits occurring side by side at the same level on the wall of one tracheide (Fig. 10).

In branch (1) and branch (2) the pits are usually quite free from one another, very rarely touching or slightly compressed. They are however much more abundant at the ends of the tracheides. In root (1) the pits, while generally free, are often in close contact, especially at the cell endings, the result being that their shape is oval with flattened upper and lower sides. The pits are sometimes flattened in root (2), but the structure is not well enough preserved to institute comparisons.

Bordered pits in Coniferae usually show two concentric circles in surface view, the outer and inner borders. It has been customary to determine the width of these circles most accurately in the hope of thus obtaining data for the separation of different plants. From the extended researches of Kraus[1], Wille[2] and others, it does not seem that there has been much progress made in this direction.

In the present specimens much difficulty has been experienced because of the varying state of preservation. At first this caused a good deal of unnecessary labour. It was found that in parts of some sections the pits showed more than two concentric circles (Fig. 15); in other cases the two circles could hardly represent the outer and inner borders of the pit, because of the large and varying size of the inner one. The appearances referred to evidently point to different stages of decomposition, some of them closely resembling for instance those figured by Hartig in his description of

[1] Kraus, Beiträge zur Kenntniss. fossil. Hölzer, II, Zur Diagnostik des Coniferenholzes, Abhandl. d. naturf. Ges. zu Halle, xvi, 1886.

[2] Wille, Zur Diagn. d. Coniferenholzer, Halle, 1887.

the decomposition of spruce-timber by *Polyporus borealis*[1]; and the far from rare occurrence of hyphae in the specimens shows that some fungus had been at work before petrifaction. The case referred to is exactly analogous to the decaying bordered pits of a piece of rotten *Sorbus Aucuparia* described by Kraus, where the inner circle increased in diameter according to the stage of decay until it fused with the outer[2]. What at first sight was regarded as a remarkable variability in the bordered pits was thus found to be due to the state of preservation of the specimen, and all measurements made in such parts were subsequently disregarded.

The inner pore, which is circular, is not usually well preserved in the specimens. The average of a number of measurements in the best places was 3–5 μ, these limits being rarely passed.

More detailed results were obtainable with the outer border. Many hundreds of measurements were taken with a view to determine whether, as in recent woods, any clearly marked increase could be noted in the size of the pits from the pith outwards. The small pits in the summer wood were left out of consideration.

The results are as follows:—

Greatest diameter of Bordered pits.

Branch (1), 3rd ring, 7·0 μ; 5th, 9·5 μ; 6th, 11·3 μ; 7th, 14·0 μ.
Branch (2), 3rd ring, 8·4 μ; 4th, 10·5 μ; 5th, 12·8 μ; 8th, 14·0 μ.
Ditto in another section, at intervals, 9·7, 10·3, 11·1, 12·0, 12·2, 13·7 μ.
Root (2), successive rings, 10·5, 13, 13, 13·3, 13·2, 12·5, 12·7, 14, 13·5 μ.
Root (2), successive regions, 10·4, 12·2, 12·3, 13·6, 13 μ.

These figures show how incompletely a single number would suffice to express the size of the pits. From our knowledge of their varying size in different organs, and in the same organ at different ages, we should expect to

[1] Hartig, The Diseases of Trees, English Edition, Fig. 124.
[2] Kraus, Mikrosk. Unters.

find a series of measurements in every recently described fossil, where the size of the specimen and the state of preservation allowed of this. Yet I have only met with one instance in which such a series of observations has been made [1].

The average diameter of the bordered pits in young roots and branches cannot be definitely stated. In the oldest parts of our sections it may be given as 13–14 μ [2].

Bordered pits on the tangential walls are present in all the sections, both radial and tangential (excepting in root (2), where the structure is not well preserved). According to Strasburger, tangential pits are found in the summer-elements of all Coniferous woods which do not possess 'tracheidal elements' in the medullary rays [3]. The function of the latter is to supply a radial passage for the water, and the tangential pits accomplish this from one ring to another, there being no room in the last summer-elements for the radial pits. In support of this view Strasburger shows that tangential pits occur in other parts of the ring where the normal passage of water from one tracheide to another is interrupted, e.g. when a new medullary ray is formed, or opposite a resin-duct. A good example of this is seen among fossil woods in *Pinites Ruffordi*, where a solitary tangential pit is figured—opposite a resin-duct [4].

In *Cupressinoxylon*, with its purely parenchymatous medullary rays, one would look for tangential pits in all the 'species.' But they have only been recorded in six of the sixty species summarized by Beust [5]. A certain amount of perseverance is necessary to find the pits in some of our sections, although they are very well seen in the others. They are much smaller than the radial pits.

[1] Conwentz, l. c., p. 40.

[2] Kraus, Zur Diagn., gives 15 μ + as the usual size in Abietinae and Cupressinae, the elements of young parts being smaller. Schenk, in Zittel, states that the full size is generally reached during the first ten years.

[3] Strasburger, l. c., p. 9.

[4] Seward, l. c., Fig. 6.

[5] Beust, l. c.

Measurements of Tangential Pits.

Branch (1). Average of 36, outer diameter 5 μ, inner 2·35 μ.

Branch (2). Presence easily noted, but not sufficiently clearly for accurate measurements.

Root (1). Average of 26, outer diam. 7·3 μ, inner 2·3 μ.

Root (2). Structure of wall not well preserved: tangential pits not met with.

The numbers of rows of summer tracheides in each ring bearing tangential pits have been carefully determined for recent Conifers, and the different groups are seen to vary considerably in this respect. Kraus recommends observations in transverse sections, but it is only possible in our sections to use the radial ones. In the latter the tangential pits are sometimes very clear. The section of the pit has the form of a sharply cut lens-shaped space, and not infrequently the torus is found stretched across it as a short black line. In other cases this space is without the torus. In a third set of examples the lumen of the pit is filled up by infiltration, but the torus is well seen as a short black line in the thickness of the wall (cf. Figs. 11–13). These three conditions are often found close together, all manner of transitions being seen. It becomes quite possible then, after careful study, to make out the tangential pits in comparatively thick sections with the help of a substage condenser. This has been of the greatest service in counting the number of rows of summer-tracheides with these pits, because this part of the wood is generally very dense.

The observations have been most successful in branch (2). Here the tangential pits are sometimes found in the last seven rows of tracheides. This abundance of tangential pits is in agreement with the other characters of our fossil wood, showing us that in the Cretaceous period, where *Cupressinoxyla* are first met with[1], the type of wood found in *Cupressus*, *Sequoia* and *Abies* was developed in its minutest details. In Abietinae with composite medullary rays these

[1] Schenk in Zittel, l. c.

pits are much less abundant, and are rare or absent in the *Pinus* group.

The torus, so well seen in certain tangential pits, is still more clearly defined in the larger pits on the radial walls (Figs. 11 and 12). These, examined in tangential sections, frequently show the torus as a dark line suspended in the lens-shaped cavity of the pit or closely applied to one side[1]—positions which might lead to interesting speculations as to the condition of the wood at the time of petrifaction[2].

Medullary Rays.

The medullary rays figure conspicuously in every published description of fossil Coniferous wood. While in Dicotyledons they are composed of similar elements but differ widely in form, in Conifers they are very uniform in shape but differ greatly in their component parts. This has led to a minute study of them in the latter class of woods. In the first place there is their difference of composition. Some of them have tracheidal as well as parenchymatous elements, and this difference is regarded as an excellent diagnostic character. Those woods further which possess vertical resin-ducts usually also have radial ones in certain of the medullary rays. The latter are then compound and are more than one cell broad. In living Conifers which have no resin-ducts, the medullary rays are simple and consist of a single vertical layer of cells. The two-rowed medullary rays, although occasionally met with, are rare[3]; but they are much more frequently found in *fossil* woods without resin-ducts, and this constitutes one of the differences between recent and fossil species[4].

[1] The torus is well seen also in *Sequoia canadensis*, Schrtr., and *Pinus succinifera*, Conwentz.

[2] Strasburger, l. c., p. 32; also Russow, Zur Kenntniss des Holzes, insbesonderheit des Coniferenholzes, Bot. Centralblatt xiii, 1883, p. 37.

[3] Beust, l. c.

[4] Kraus, Mikr. Unters., mentions *Cupressinoxylon fissum*. Araucarian woods with two-rowed medullary rays are called *Pissadendron*. Among woods with resin-ducts, Strasburger mentions *Larix* as not infrequently having double medullary rays without resin ducts.

In the fossils under examination the medullary rays are uniform in structure, consisting of parenchyma alone; they are also generally one cell thick (excepting in root (2), which should perhaps be separated because of this and other differences in the medullary rays).

The characters mentioned thus far are of great importance in descriptions; as regards the constancy of those to follow we are a good deal in the dark. The earlier writers, in describing the medullary rays, were content with mentioning whether they were simple or compound, and indicating the height they might reach in cells vertically above one another, e.g. 1–18, 1–6, 2–36, &c. But the uselessness of these numbers will be at once apparent to any one who has examined a number of tangential sections of Coniferous wood, the maximum height of the medullary rays depending largely on the time taken in their examination. It has moreover been demonstrated that in this respect medullary rays vary according to their distance from the pith and height up the stem; and stems, branches and roots of the same plant may have medullary rays of different heights[1]. Nevertheless we do meet with cases where the height of the medullary rays is more or less characteristic of the wood. They are usually low in *Thuja*, higher in *Cupressus* than in *Juniperus*, in Abietinae they have half the number of cells they have in Cupressinae (except *Thuja*), &c. It is therefore necessary to include the height of the medullary rays in all diagnoses, especially in those cases where we have a knowledge of the age of the part and its morphological value.

A similar series of remarks is applicable to almost every one of the characters of medullary rays which different writers have from time to time attempted to introduce. Their frequency in the wood, the height and width and radial length of the individual cells, the thickness and pitting of the walls and the general shape in cross-section—all these have been demonstrated to be valueless for the

[1] Essner, Ueber den diagn. Werth der Anzahl u. Höhe der Markstrahlen bei den Coniferen. Abh. d. natur. Gesell. zu Halle, xvi, 1886.

general separation of species, and yet there are certain genera which show marked and apparently constant peculiarities in one or other of these respects. A careful analysis is therefore necessary of the medullary rays and their cells. Such has been attempted in our sections, following as far as possible the lines laid down by Kraus, Essner, and Beust in their investigation of living forms.

At the instigation of Kraus, Essner has shown that the numbers of medullary rays per sq. mm. of tangential section in any species is greatest in the first year, and that it decreases gradually outwards, while on the other hand the medullary rays are lowest near the pith and increase in height towards the cortex. This has led him to the idea that the number of medullary cells per sq. mm. may be constant for the same species in different years. In his attempts to obtain characteristic numbers for different species, he found, however, that individuals of the same species varied more than did those of different species. This character cannot therefore be considered absolute.

Beust, arguing from the intrinsic importance of the medullary-ray-tissue in the life of the wood, regards it as probable that some relation exists between the relative bulk of this tissue and that of the tracheides. To obtain this proportion he has combined the several factors determined by Essner. It is however not certain at present how far the proportion will hold good for the different types of wood.

I have attempted to apply these observations to the fossil sections. It is not easy to determine accurately the age of the part in a tangential section of a fossil. In my specimens they have been cut as a rule between the sixth and the tenth rings of growth. The following figures represent a great deal of labour. Their worth will not be fully apparent until it is possible to compare them with those of other specimens. As their value greatly depends upon the number of medullary rays observed, I have where possible noted this.

The first (p. 357) table gives the number of medullary rays per sq. mm., as well as their heights in cells. All the slides here examined were tangential. The method adopted was to count the number of medullary rays in the field of the microscope, the area of the field having first been accurately determined. In the case of medullary rays partly included those only were reckoned which were more than half in the field.

The second table (p. 358) gives the number of medullary-ray-cells per sq. mm., as well as the average area of each cell. These combined give the proportion of medullary-ray-tissue to the whole wood. The observations on the radial sections are given as control measurements. The areas are calculated from the tangential measurements alone. Each medullary-ray-cell is practically an ellipse. In measuring its width it was impossible to decide how much of the cell wall belonged to the cell of the ray and how much to the adjoining tracheides. The whole thickness of the wall on one side was therefore added to the lumen.

Pits were observed only on the radial walls of the medullary-ray-cells. In both radial and tangential sections these were seen to be simple. In shape they were oval and obliquely placed, usually two or one per cell, occasionally three, or more rarely four. The higher numbers were, as usual, on the outer cells, the middle cells having frequently one pit each. This is in accordance with the *Sequoia* type of wood.

The longest diameter of the medullary-ray-pits could only be accurately determined in two sections. In branch (1) it varied from 3 to 10 μ, the average of fourteen pits being 6·5 μ. In root (2) the diameter varied from 6 to 12 μ, the average of thirteen pits being 8 μ.

Intercellular spaces were very clearly seen in the medullary rays, their distribution being as in recent woods.

Resinous Parenchyma.

There are no resin-ducts. Wood-parenchyma is however to be found among the tracheides, and this, from analogy

TABLE I.

		No. of medullary rays counted	Height of medullary rays in cells, showing the number of medullary rays of each height in every hundred examined †.											Highest and rarest medullary rays observed.	Average number of cells per medullary ray.	No. of double medullary rays.	Total number of medullary rays examined.
			1	2	3	4	5	6	7	8	9	10	11				
Hr. 1	Section 1*	62	13	38	22	13	8	3	3	...	...	...	...	1-7	2·96	...	124
	Section 2	57	9	43	23	12	6	3	3	...	1	...	...	1-9	2·90	...	113
Hr. 2	Section 1	94	23	33	13	14	5	5	5	...	1	1	...	1-15	2·93	1%	256
	Section 2*	94	30	53	16	7	2	2	0	...	...	...	...	1-6	2·24	...	195
Rt. 1	Section (1)	58	38	43	14	4	1	...	...	...	...	...	...	1-5‡	1·87	...	131
	Section (2)	53	30	47	12	5	3	...	...	...	...	...	...	1-5	2·04	...	307
Rt. 2	Section 1	84	17	34	17	12	9	6	3	1	...	1	...	1-11‡	3·06	3%	837
	Section (2)	75	7	37	16	14	11	5	3	2·5	2·5	1	1	1-16	3·54	4·5%¶	586

* In this section only a small portion was accurately tangential, therefore all the medullary rays were obtained from one part of the section.

† Very instructive curves may be prepared embodying these figures, the numbers of medullary rays of different sizes being represented by the height of the curve above the base-line. In parts near the pith the curves are steepest, becoming rapidly flattened as we proceed outwards.

‡ Felix gives low medullary rays as characteristic of roots[1]. This agrees well with root (1), but not with root (2), making it possible that the latter may have to be separated.

¶ In one part of this section twenty eight double medullary rays were counted in fourteen fields, i.e. 9 %, one being three cells broad. They may be frequently seen in transverse sections of this specimen.

[1] Felix, Inaug. Diss. 1882, &c.

with recent woods may be regarded as resiniferous[1]. The resiniferous parenchyma may be described as abundant, and our wood is therefore to be placed under *Cupressinoxylon.*

In a transverse section of recent Coniferous wood the parenchyma may frequently be determined by its contents or the thinness of its walls, and its relative abundance thus

TABLE II.

		No. of medullary ray cells per sq. mm.	Size of individual cells † in μ (about fifty measurements in each case). Height (radial sections).	Height (tangential sections).	Width.	Average area of cells in μ².	Amount of medullary ray tissue per sq. mm.	Proportion of medullary ray tissue in wood.
Br. (1)	Section (1)	183	(17, 18)	16	14	176	·032 mm²	1 : 30
	Section (2)	165		19	14	209	·034	1 : 28
Br. (2)	Section (1)	281	(16·8, 15·6)	15	12·7	151	·042	1 : 23
	Section (2)*	211		15·7	14	173	·037	1 : 26
Rt. (1)	Section (1)	108	(18·5, 21)	19·6	16	246	·027	1 : 36
	Section (2)	111		20·5	16	257	·029	1 : 33
Rt. (2)	Section (1)	270	(18·8)	19	14·2	212	·057 ‡	1 : 17
	Section (2)	271		19	14·2	212	·057	1 : 17

* See note to Table I.
† The radial length varied from 23 to 159 μ and covered from 2 to 6 tracheides.
‡ See note to Table I.

noted. This is not possible in our specimens, where the walls of adjoining cells are not separable, and it is difficult to distinguish the resin-cells from tracheides with dark contents. In longitudinal sections, however, the contents of the parenchyma are seen to be peculiar, and differ from the small rounded granules found in the tracheides. Spherical

[1] Strasburger, l. c.

masses of dark substance give the parenchymatous cells a vacuolated appearance[1]. This peculiarity has been of great service in the search for parenchyma in each section (cf. Figs. 8 and 9). Transverse walls are always found in the immediate neighbourhood of such masses, as if the contents had accumulated here during the disintegration of the wood; and these transverse walls alone determine the parenchyma. There is thus some reason for the frequent description among the older writers of 'fossil resin' and 'fossil starch.'

The strands of wood-parenchyma occur as isolated rows of cells separated by transverse walls. In tangential sections the terminal cells of each row have pointed free ends, whereas in radial sections the end cells appear to terminate abruptly at a medullary ray just as many of the tracheides do. This suggests that each strand has an origin similar to that of a tracheide—from a single cambial cell. Occasionally several strands appear close together, and they are more abundant in certain sections or parts of sections. But generally speaking, the resiniferous tissue may be described as uniformly distributed.

The cells differ in appearance in radial and tangential sections. In the latter the transverse walls cut the cells across at sharp angles, sometimes slightly obliquely, and the whole strand is of equal width excepting at the ends (Fig. 9). In the radial view, on the other hand, the transverse walls are never oblique, the cell-lumen is rounded at each end, and there is usually a distinct constriction in the width of the cell at each division (Fig. 8). These facts are of considerable importance. In the first place, the shape of the wood-parenchyma-cells has been regarded as of great value by some writers[2]; and secondly, various species have been characterized as having rows of cells constricted at intervals. Andrae, in an all-too-short description of *Calloxylon Hartigii*—a *Cupressinoxylon*

[1] This vacuolated appearance has been noted by Kraus, Mikr. Unters., and drop-like masses of a similar nature have been described by Andrae.

[2] Hartig, according to Kraus, Mikr. Unters.

not unlike our own in several respects—founded on the examination of over a hundred sections, noted the same characters in the radial and tangential aspects of the wood-parenchyma [1].

There is a marked difference in the width of the cells in radial and tangential directions. The radial widths in the different specimens were 19 μ, 11 μ, 20 μ, and 19 μ, respectively, whereas the tangential were 28 μ, 19 μ, 26 μ, and 26 μ. The length of the cells varied from ·09 mm. to ·33 mm. in branch (1), with an average of ·19 mm. in twenty-five measurements; in root (1) from ·04 mm. to ·21 mm., with an average of ·12 mm. in twenty measurements; in root (2) from ·11 mm. to ·27 mm., with an average of ·19 mm. in twenty measurements.

The terminal cells of each row of parenchyma are, as already stated, pointed in tangential sections, and are here indistinguishable from the neighbouring tracheides. These end-cells are free of contents and appear to have normal bordered pits. The latter are however difficult to make out. It is quite possible that the appearances are deceptive, because Strasburger states that in recent Coniferous woods the pits between tracheides and parenchyma are always one-sided bordered pits [2]. See, however, Fig. 14.

In one case a strand of resin-parenchyma was traced through 2·5 mm. when the edge of the section was reached, nine transverse walls having been passed. The average length of ten closed series of parenchymatous cells from end to end was 1·55 mm.

It was not possible to make out any pittings on the transverse or tangential walls of the parenchyma. This is one of those subsidiary characters which should always be noted, for it is stated by Beust that the wood of *Taxodium*

[1] Andrae, *Calloxylon Hartigii*, Bot. Zeit. 1848, p. 633. See also Kobbe, l. c., Taf. ii, figs. 1 and 3 of *Cupressinoxylon uniradiatum*.

[2] Strasburger, l. c. See also Kobbe, l. c., where double-bordered pits are figured in the parenchyma of *Cupressinoxylon uniradiatum* (Taf. ii, fig. 1) and of *Cupressinoxylon pachyderma* (Taf. ii, fig. 10).

distichum and ***Thuja gigantea*** may be distinguished from all others by the thick pitted transverse walls of these cells. The reticulated pitting on the tangential walls of ***Thuja gigantea*** and ***Fitzroya patagonica*** is also considered a good character.

EXPLANATION OF FIGURES IN PLATES XXIII AND XXIV.

Illustrating Mr. Barber's paper on *Cupressinoxylon*.

Fig. 1. Transverse section of branch (1) of ***Cupressinoxylon vectense***, showing composite rings. × 3½.

Fig. 2. Transverse section across composite ring of a branch - the same specimen as in Fig. 1—showing gradual and sudden transitions between the large and small elements. × 60.

Fig. 3. Transverse section across part of a root. × 30.

Fig. 4. A partial ring which disappears completely to the right. × 30.

Fig. 5. Part of the medullary sheath of branch (1). The rows of tracheides terminate towards the pith in small-celled groups. This is better seen in the portion more highly magnified in the next figure. × 45.

Fig. 6. Part of the medullary sheath shown in Fig. 5, more highly magnified. × 100.

Fig. 7. Pith of root (2). The rows of tracheides pass uninterruptedly into the pith. There is no appearance of the groups of small cells representing the protoxylem. × 100.

Fig. 8. Resiniferous parenchyma in radial section with drop-like masses of fossil resin. × 350.

Fig. 9. Resiniferous parenchyma in tangential section. × 350.

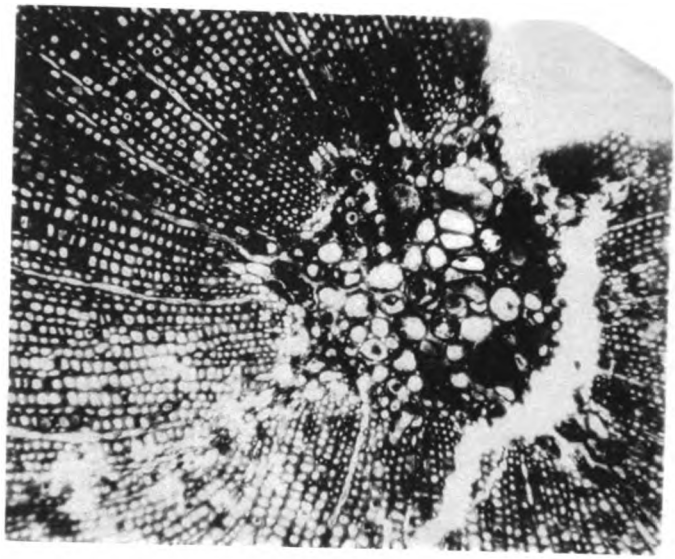
Fig. 10. Tracheide showing the occasional doubling of the row of bordered pits. × 500.

Figs. 11 and 12. Walls of tracheides in tangential section showing the cavity of the pits with suspended torus. × 500.

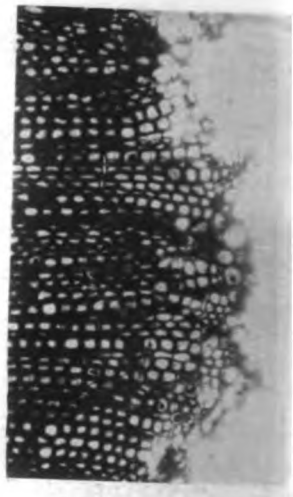
Fig. 13. Radial section through part of the summer-wood of a composite ring with the torus preserved as sharp dark lines. All stages are met with, frequently near together, between the appearances in Figures 11, 12, and 13. × 500.

Fig. 14. Resiniferous parenchyma in tangential section. The dark oval bodies in the walls appear to be small double-bordered pits. × 800.

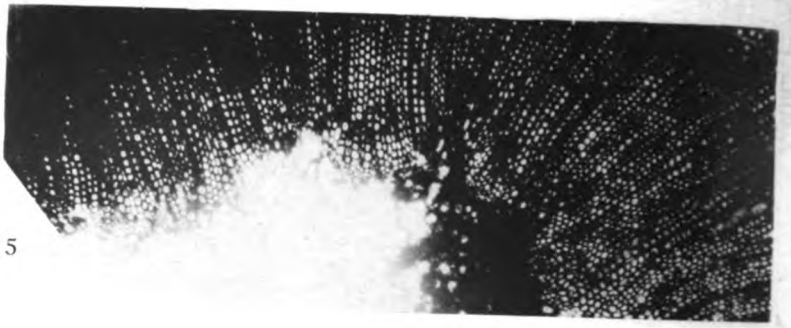
Fig. 15. Various appearances presented by bordered pits in surface view, probably depending upon the state of preservation. × 750.



7

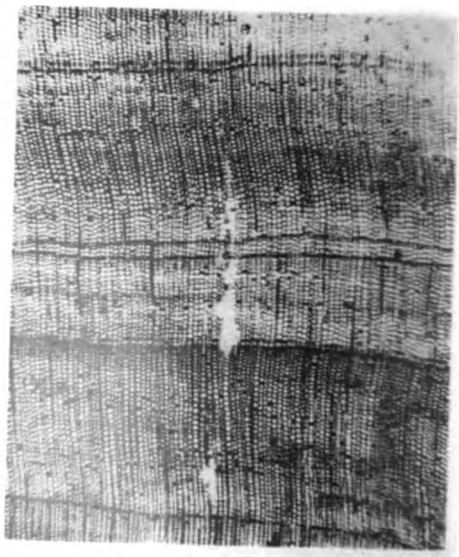


6

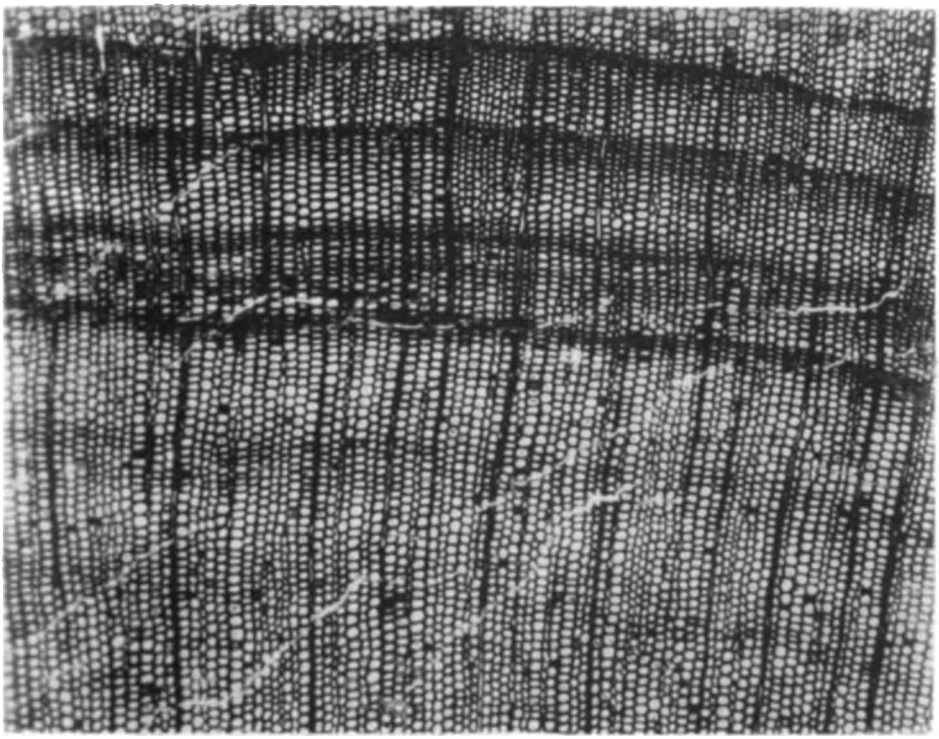


5

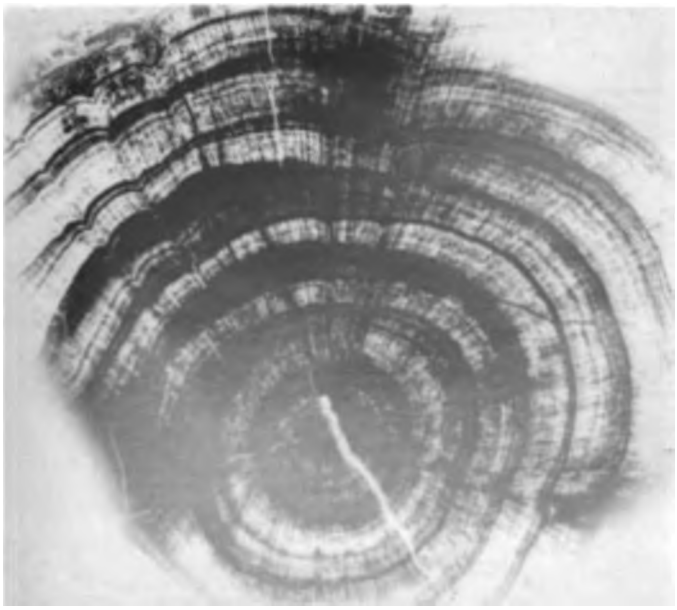
4



3

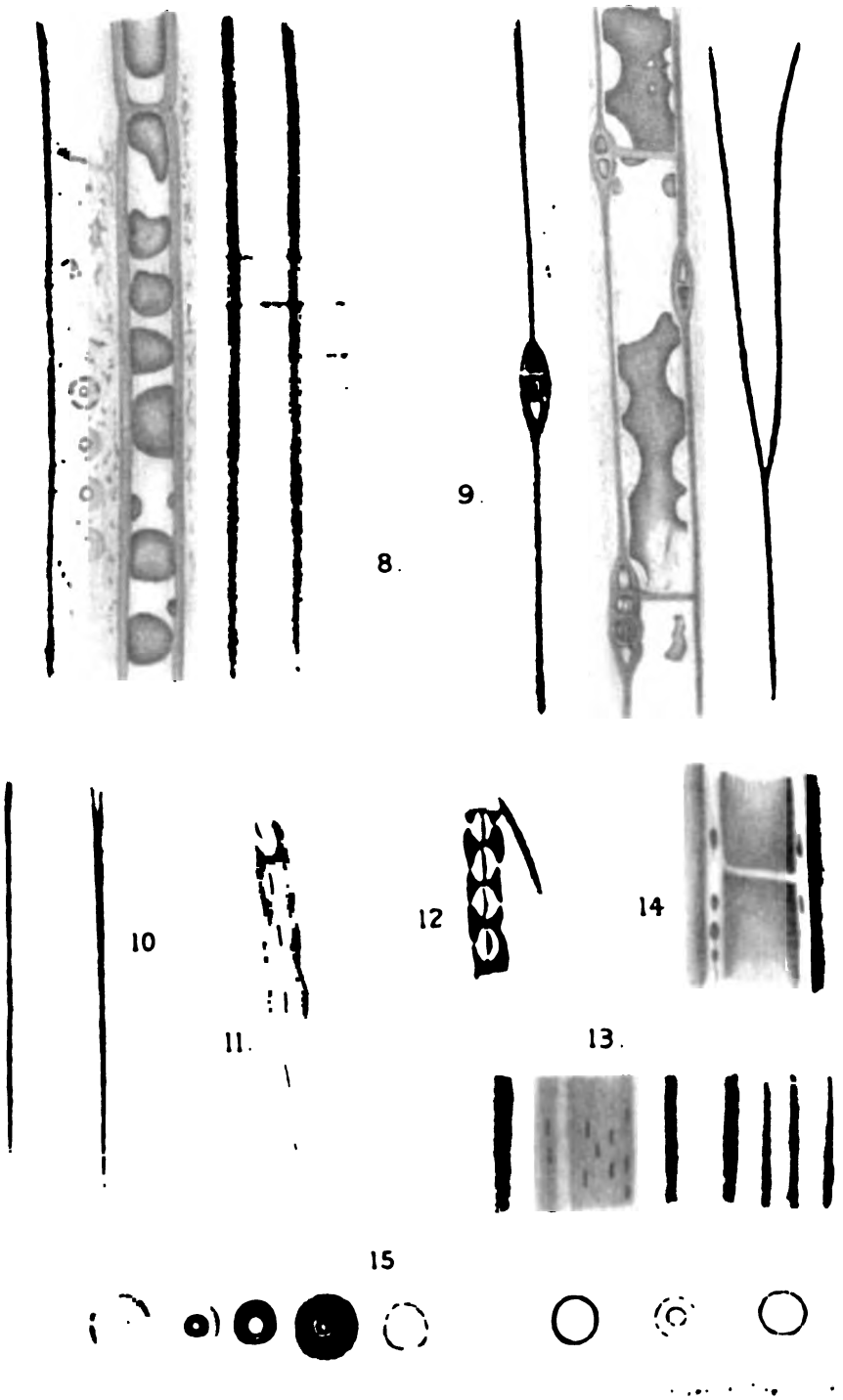


2



1

University Press, Oxford.



BARBER CUPRESSINOXYLON

The Action of Cold and of Sunlight upon Aquatic Plants.

BY

ALFRED J. EWART, D.Sc., PH.D., F.L.S.

IN a recent paper by Messrs. West and West, these authors take exception to certain results published by me two years ago in my first paper upon Assimilatory Inhibition [1]. The objections which they make form an admirable illustration of the mistake into which biologists and collectors often fall in making use, or rather misuse, of experimental observations made by the physiologist in the laboratory. The prejudice which seems to exist in certain quarters against the results of laboratory investigations is an extremely absurd and unreasonable one, for it is only by research of this kind that any accurate and precise knowledge of fundamental principles can be obtained. The conditions under which plants exist in nature are so variable and complicated that it is impossible by direct observation to determine precisely what are the factors engaged in producing any given result, especially since any marked change in the external conditions must necessarily affect not merely one, but directly or indirectly every form of vital activity of which the plant is capable. It is true that particular

[1] West and West, Ann. of Bot., 1896, Vol. xii, p. 33; Ewart, Journ. Linn. Soc. Bot., Vol. xxxi, 1896, p. 217.

[Annals of Botany, Vol. XII. No. XLVII. September, 1898.]

influences may directly affect single functions only or these in more marked degree than in others, but in every case, even if only a single function is directly affected, an indirect effect must finally be produced upon all the other functions, provided the stimulus affecting the first function acts for a sufficiently long period of time. This is a necessary corollary to the intimate connexions and correlations which exist in the living organism between its different functional activities.

In laboratory experimentation we make use of these principles, the effects produced on single functions being noted in plants kept under otherwise normal conditions, while at the same time the direct and indirect effects produced are as far as possible distinguished from one another. Having obtained a knowledge of the action of the different factors constituting the external world upon each particular vital function, the next thing necessary is to submit the results thus obtained to the test of nature, by which they must stand or fall. If, however, the observations made in nature do not coincide with the results obtained by laboratory experiment, it does not necessarily follow that either are incorrect. The discrepancy between the two may simply indicate that in either or both cases certain operating factors have not been noticed or allowed for.

It is therefore as absurd for the biologist to deride the results obtained by laboratory experimentation, as it would be for the physiologist to disregard the criticisms and limitations which the biologist imposes upon him. Only by giving to each their proper value and importance can the facts observed by the biologist and physiologist be correlated and brought into harmony with one another[1].

Bearing these facts in mind, I proceed to discuss certain points in the paper by Messrs. West, to which the above disquisition appears to be especially applicable. In the

[1] An admirable general account of these and similar questions is given in the introduction to Pfeffer's new Pflanzenphysiologie. (An English translation is in process of preparation for the Clarendon Press.)

first place, Messrs. West are able, by citing a fragmentary sentence from my paper on Assimilatory Inhibition, to quote on my apparent authority the statement that no fresh-water Algae are resistant to cold, and that all are killed by being frozen. It is perhaps hardly necessary to mention the fact that the statement made was expressly intended to refer only to those Algae actually examined. These were extremely few in number, for the above research was conducted solely in order to determine the influence of various normal and abnormal injurious agencies upon the function of CO_2-assimilation. Moreover, for this very reason, the plants employed were such as were in an actively vegetating condition, and which had been grown or had been kept for some time previously under optimal conditions of temperature, &c. It is well known that many plants can be gradually accustomed to low temperatures (or other injurious agencies) to which, when suddenly exposed, they inevitably succumb. Frequent reference is made to this long-known and frequently studied peculiarity throughout the work mentioned and in subsequent ones also. In the work first published, the only fresh-water Algae examined with regard to the effects of cold upon them were undetermined species belonging to the genera *Oedogonium* and *Spirogyra*, and two to that of *Cladophora*. To have founded any general conclusions as to the resistant powers to cold of the great group of fresh-water Algae upon such isolated observations as these, would indeed have been somewhat premature. It is however certain that many, or perhaps very many or all, of the higher filamentous fresh-water Algae, and perhaps of other aquatic plants as well, are exceedingly sensitive, when in the actively vegetating condition, to temperatures approaching to or falling but little beneath the freezing-point. A few observations recently made may be of interest here, for they illustrate more precisely what the resistant powers to cold of such plants actually are. In all cases the plants employed were actively vegetating, and had been kept at a temperature of from 15° to 20° C. for

some time. That *Spirogyra* is very sensitive to cold is quite certain. Thus a sample of class-material composed of *S. crassa* and *S. nitida* was killed in a single night, owing to the temperature accidentally being allowed to fall from about 20° C. to just below zero, and that although no actual ice-crystals were formed in the water in which the plants lay. Similarly with actively vegetating specimens of *Spirogyra nitida*, *S. crassa*, *S. flavescens*, *Vaucheria sessilis*, *V. terrestris*, and of *Nitella*, all the vegetative parts were killed by exposure during a single night to a temperature not falling below −2° to −3° C., which caused a partial conversion only of the surrounding water into ice. After freezing for one night at from −2° C. to −5° C., *Vallisneria spiralis*, *Cladophora*, and *Chara* were entirely killed; in *Elodea canadensis* occasional living cells were still to be found; *Lemna minor* and *Lemna trisulca* were partially killed; while of Diatoms and Desmids, very many of the Desmids *Euastrum* and *Closterium* were killed, and many of the Diatoms *Navicula* and *Pinnularia*. After being frozen at −8° C. to −10° C. for several hours by means of a freezing mixture of ice and salt, *Lemna minor* and *Lemna trisulca* were killed, as well as both the Diatoms and Desmids. In the former, as is usually the case, the chlorophyll bodies were broken up and disorganized. In the latter the 'Endochrome' was also disorganized and frequently shrunken, while no plasmolysability could be detected in any of the cells. When, however, the cultures were returned to normal conditions, after two weeks or so, living Diatoms of the same kind reappeared, but no Desmids. In the former case apparently a few resistant forms had remained living and escaped observation. This might possibly have occurred with the Desmids also had these previously formed any resistant spores.

Of several Oscillarias examined, all remained living, though in some cases the filaments were in part killed. Similarly in *Gloeocapsa*, *Scenedesmus*, and *Protococcus* most or almost all of the cells remained living, whereas the zoospores of *Protococcus* (*Chlamydococcus*?) were dead and non-motile when

thawed out[1]. It is interesting to notice that, when in the motile condition, organisms appear to be more sensitive to cold (and to other injurious agencies as well) than when in the non-motile resting or vegetating condition. This may be partly due to the protecting cell-wall being thin or absent in the motile condition, partly to the more highly developed functional activities and special irritabilities which the motile zoospore possesses. In the motile and highly irritable condition the plant is able to avoid injurious agencies to a greater extent than it can when in the non-motile or fixed condition, and hence, in the former case, a development of high resistant powers is less necessary; for the motile stages are, as a general rule, of only short duration, and are produced only when the external conditions are favourable.

Even in multicellular plants, the protoplasts of which are always clothed with a cell-wall, an increased motility of the plasma seems to go hand in hand with a decreased resistant power to cold. In certain cases, at any rate, an increase in the amount of water present in the cell or cell-plasma accompanies this increased motility. This is the case, for example, in the rotating cells of *Chara* and *Nitella*, in the staminal hairs of *Tradescantia*, and in the parenchyma of the leaf and stem, and in the epidermal cells of *Elodea canadensis* and *Vallisneria spiralis*, in which various stimuli readily induce rotation. For in all these cases streaming movements appear only as the amount of water present in the cell increases, and the most active rotation is shown only when a single large central vacuole is present. No cell, the protoplasm of which shows active streaming movements or rotation, appears to be able to withstand either freezing or desiccation[2].

From the observations made by Messrs. West upon the survival of certain forms in frozen pools from year to year,

[1] Zoospores of *Ulothrix* and *Haematococcus* can continue to move at 0° C.

[2] See Assim. Inhib., Journ. Linn. Soc. Bot., Vol. xxxi, p. 394; also Trans. Liverpool Biol. Soc., 1897, p. 157.

although no formation of zygospores was observed in them, no precise conclusions can be drawn. It is sufficient to recall how frequently the existence of special reproductive organs in fresh-water and marine Algae has been overlooked by a series of investigators to make certain how unreliable any such negative observations are, however carefully conducted they may be. The most painstaking and conscientious investigator would shrink from the task of subjecting to thorough and systematic microscopical examination the entire contents of even a small pond filled with vegetable life, and yet, as is well known, the existence of only a few zygospores might cause, under renewed favourable conditions, the original vegetation to be restored in a comparatively short period of time. Moreover, a formation of asexual aplanospores or of resting-cells might escape observation with even greater readiness. That, however, the zygospores, aplanospores, and resting-cells may remain dormant for prolonged periods of time is well known, and that they can resist considerable extremes of temperature, such as would be immediately fatal to the plants when in the actively vegetating condition, is extremely probable, and indeed almost certain from the observations which Messrs. West record. It must, however, always be remembered that at the bottom of the pond, in the mud, &c., in which the spores lie, the temperature is not necessarily and indeed is rarely the same as it is on the frozen surface.

Nor does it follow that plants imbedded in ice or covered by snow are frozen. Both snow and ice are very bad conductors of heat, and therefore, as is well known, a layer of snow acts as a protective mantle to the plants which it covers. Living plants have a power of producing heat which may be so marked, when no transpiration is going on, as to raise their temperature several degrees above that of the surrounding medium. Beneath the snow, however, this factor hardly comes into play, since at low temperatures the vital activities of even the most resistant plants are reduced to a minimum or to nil, and it is upon the con-

tinuance of these vital activities that the production of heat depends. Thus cell-division ceases to take place at zero or a few degrees above it[1]; CO_2-assimilation ceases in tropical plants between 4° C. and 8° C., in warm, temperate, sub-tropical and water-plants between 0° C. and 2° C., while in cool, temperate, arctic and alpine plants assimilation only ceases when the plants are frozen, i. e., at a few degrees below zero[2]. Similarly rotation gradually diminishes as the temperature falls below the optimum, and after remaining for a prolonged period at a temperature at or near zero may cease, temporarily or permanently[3], while faint respiration may go on even below zero until a temperature of −10° C. is reached[4]. It is evident that even when a plant is surrounded by a medium the temperature of which is several degrees below zero, since faint respiration may, in very resistant plants (many Conifers, Mosses, Lichens, Schizophyta, and unicellular Algae), still continue, the interior of a morphological or accidental cell-aggregate may be at a slightly higher temperature, provided the loss of heat by conduction, radiation, or transpiration is slight or absent. Though a trifling, this is by no means a negligible quantity, and may, since the production of heat goes on continually, be one of considerable importance, when all transpiration is prevented and the plant is frozen in ice and surrounded by an insulating jacket of air, or is covered by snow. Fresh-water plants, especially if massed together, are frequently found to be covered by bubbles of gas when the water in which they lie freezes. Moreover, the water around the plant may be melted or absorbed so that the bubbles coalesce, and the plant or portions of it come to lie in insulating air chambers, which form an admirable protection from the outside cold. In such a chamber, when exposed to ordinary light, the heat-rays which penetrate the ice will

[1] Strasburger, Zellbildung und Zelltheilung, 1880, p. 171.
[2] Ewart, Assim. Inhib., Journ. Linn. Soc. Bot., Vol. xxxi, p. 401.
[3] Cf. Ewart, l. c., p. 394.
[4] Jumelle, Rev. Gen. de Bot., Vol. iv, 1892, p. 114 seq.

be collected and concentrated upon the plant by the concave walls of the chamber in which it lies, and thus may keep the temperature of the plant from falling below its own special freezing-point during the daytime. The chlorophyll, along with the water, &c., which the plant contains, forms a very efficient warmth absorbing agency. It is probably by means of the heat thus directly absorbed that the ice immediately surrounding a frozen plant may be melted, even when exposed only to the relatively feeble rays of the wintry sun. I have frequently found the vegetative shoots of *Elodea* enclosed in ice in this manner, and have seen in some cases that the stem and most of the leaves were in part or entirely living, although the vegetative shoots of this plant are always infallibly killed when properly frozen. In plants covered by not more than two or three centimetres of snow, radiation from above cannot supply any appreciable amount of heat to the plant, owing to the relatively high opacity of loose or freshly fallen snow when in mass both to radiant light and heat. Nor can any noticeable amount of heat be derived by the plant under these circumstances by radiation or conduction from beneath. The special efficiency of snow as a protective agency seems to be simply due to the fact that the mixture of air and ice of which it is composed forms an admirable insulating medium so long as the temperature remains low, while at the same time the loss of heat by transpiration or by radiation is reduced to nil or to a minimum.

The case mentioned by Messrs. West, in which filaments of *Spirogyra* were found, when thawed out from the ice in which they had been frozen for some time, to be in conjugation, and that nevertheless the vitality of the filaments was unimpaired, is hardly applicable to the point at issue, and moreover, involves two contradictory statements. Surely in the process of conjugation the vitality of the *filament* is always impaired, for with the completion of the process the further existence of the filament ceases. Each zygospore is a new organism, and possesses very different resistant

powers from those of the vegetative filament which produced it. It contains much less water than the cells of the vegetative filament do, and its powers of resistance to desiccation and freezing are correspondingly increased. As is well known, unfavourable conditions generally tend to induce conjugation. Thus, plants of *Spirogyra* may be caused to conjugate by allowing the water in which they are kept to very gradually evaporate. Hence conjugation may, in many cases at any rate, be regarded as a sign that the external conditions have become unfavourable in some respect, and that the plant is endeavouring by those means to tide over the unfavourable period. This action of unfavourable external conditions in inducing a formation of zygospores is strictly comparable with the effects which partial starvation or an unfavourable habitat may exert upon many higher plants, causing them to run more rapidly to seed, and thus hastening their own exhaustion.

No haphazard observations in which the period and conditions of exposure can only be approximately estimated can afford any decisive evidence in dealing with even the simplest problems of this kind. When a perennial land-plant is subjected to gradually increasing cold, as, for example, at the onset of winter, it commonly prepares itself in a variety of ways for the increased cold which may be expected to follow. In addition to the immediately obvious external changes, certain important internal changes also take place. Thus the starch in the bark, &c., of Phanerogamic trees and shrubs, as Fischer and others[1] have shown, and in the leaves of evergreens as well, according to Lidforss, may be converted into sugar in cold winters, and be retained as such, dissolved in the cell-sap, while starch-grains reappear, after a more or less prolonged latent period, when the plant is brought into a warm room. By the above means the concentration of the cell-sap is markedly increased, so that the freezing-

[1] Fischer, Beiträge zur Physiologie der Holzgewächse, Pringsh. Jahrbücher, Bd. xxii., Heft 1, pp. 73-160: Bengt Lidforss, Zur Physiologie und Biologie der wintergrunen Flora, Bot. Ct.-Bl., Bd. lxviii, 1896. p. 34.

point of the plant as a whole is lowered, and it is enabled to withstand cold, to which, if suddenly exposed in summer, it would have been more sensitive, and by which it might have been killed.

Similarly water-plants may undergo preparatory modifications when winter is approaching. Thus in *Elodea* the vegetative shoots die down, while from the rhizomic stems buried in mud arise condensed etiolated shoots, the cells of which are packed with starch-grains in November. Later still this starch may partially or entirely disappear, being gradually converted into sugar as the cold becomes more intense. In such mud the plants are well protected, for the water it contains may not freeze until the temperature falls several degrees below zero; and moreover the chemical changes and decompositions which continue to go on to a certain extent in organic mud, even at low temperatures, may produce a slight but by no means negligible quantity of heat, and a correspondingly higher temperature. Ordinary turgid plant-cells do not begin to freeze until the temperature falls from two to four degrees below zero. As ice-crystals form and water is extracted, the freezing-point for the more concentrated watery solution remaining is correspondingly lowered. Moreover, the freezing-point for capillary imbibed water undergoes an even more marked depression, for, as Dixon and Joly [1] have shown, the imbibed water in the walls of wood vessels does not freeze until a temperature of $-10°$ C. or $-11°$ C. is reached. Hence it is only when the temperature is at least as low as this that the plant is *completely* frozen, though it commences to freeze at two or three degrees below the freezing-point for distilled water.

The statement, therefore, that no purely aquatic fresh-water Alga, or, indeed, that no purely aquatic fresh-water plant, can withstand complete freezing when in the actively vegetating condition is one which, though sufficient evidence is not yet at hand to establish the generality of its application, will

[1] Dixon and Joly, Ann. of Bot., 1895, Bd. ix, p. 416.

almost certainly be corroborated by the necessary experimental research. All plants growing entirely submerged in fresh water must be always in a condition of maximal turgidity, and contain the full amount of water possible. As is well known, it is those parts which are richest in water which are first affected by cold, and are most readily injured by being frozen. It does not, however, necessarily follow that a tissue rich in water will be killed by being frozen, as is shown by the high resistant powers exhibited by the leaves of *Sempervivum*, *Sedum*, &c., and by many Mosses as well[1].

With regard to amphibious plants, i.e. plants which (1) grow partly in and partly out of the water, or (2) float upon its surface, or (3) may lead either a submerged or a subaerial existence, these seem to have acquired more marked resistant powers than are possessed by plants growing always completely submerged in fresh water. This is only natural and to be expected, considering the variable conditions under which such plants live and the exposure to which they may be subjected; but even then comparatively few, excepting Schizophytes, such as the Cyanophyceae and Bacteria, seem able to withstand complete freezing when in the actively vegetating condition. That the plants of the first class die down at the approach of winter is familiar to all, though in cases where the water remains comparatively warm throughout the winter, the submerged leaves may remain living and green, and even form starch. Of the second class *Lemna* affords a good example, as also do the floating leaves of Water-lilies, &c. That the leaves of Water-lilies are killed by being completely frozen is a matter of common observation. *Lemna* appears able to withstand partial freezing, especially in the younger parts, but all vegetative parts are killed by being completely frozen.

The third class includes all forms which can grow indifferently in water, or upon mud, damp soil, or other

[1] See Assim. Inhib., Journ. Linn. Soc. Bot., Vol. xxxi, 1896, p. 389.

substrata. It therefore embraces *Pleurococcus*, *Scenedesmus*, *Protococcus* and allied forms, Schizophytes such as the Cyanophyceae and Bacteria, and such plants as *Vaucheria*, *Mucor*, &c. It is in this group that the forms most resistant to cold are met with, though all grades of resistant powers are shown. In this connexion it is important to notice the relationship which exists between resistance to desiccation and resistance to freezing. All plants or parts of plants which can withstand desiccation can also withstand freezing[1], though many plants which can be frozen and yet remain living cannot be fully desiccated or even completely air-dried without being killed. Thus Schröder[2] found that the leaves of *Echeveria secunda* began to die on losing from 50 to 60 per cent. of the water normally present, and died on losing 60 to 80 per cent. *Sedum elegans* gave similar results, but was able to withstand more complete desiccation. These plants and *Sempervivum* also can, however, survive exposure even to comparatively severe cold[3]. The reason for this difference appears to be that freezing involves a less complete and perfect withdrawal of water from the living cell and cell-plasma than desiccation does, and that on thawing the water is immediately available for reabsorption, whereas in desiccated specimens it is removed not merely from the cell, but away from the plant entirely. It is with regard to the capillary imbibed water that the difference between freezing and desiccation is probably especially manifest. By complete desiccation, not only all free water, but also very nearly the whole of the capillary imbibed water, may be removed; and only certain seeds, Mosses, Bacteria, &c., can withstand desiccation reducing the amount of water present to as low as from 1 to 2 per cent. When a moist plant is frozen, however, the capillary imbibed water

[1] See Assim. Inhib., Journ. Linn. Soc. Bot., Vol. xxxi, 1896, pp. 374-402; and Power of withstanding Desiccation in Plants, Trans. Liverpool Biol. Soc., Vol. xi, 1897, p. 151.

[2] Bot. Unters. Tübingen, Bd. ii, Heft 1, 1886, p. 5.

[3] See Assim. Inhib., 1896, l. c., p. 389.

does not apparently begin to freeze until the temperature approaches or falls below $-10°$ C. The water occupying the centre of the micellar canals or interstices of an organized structure will be the first to freeze. The film of water or layer of water-molecules directly abutting upon the walls of the micellar canals and coming within the sphere of the molecular influences radiating from the molecules, molecular complexes, or micellae composing these walls, will be restrained with even greater force from assuming the molecular arrangement and character constituting the solid condition. The amount of such water is, however, relatively trifling, and being firmly retained can hardly be made use of in metabolism when no other supply of water is available. It is possible, therefore, to make the dogmatic statement that no manifestation of vital activity can take place at a temperature below -10 C. to $-12°$ C. Jumelle has already been shown to be in error in supposing that CO_2-assimilation could continue at temperatures as low as $-30°$ C. to $-40°$ C.[1] It is, however, in all cases safe to conclude that plants which can withstand complete desiccation cannot be killed by cold. This has been shown to be the case with several of the more resistant Bacteria and bacterial spores by the researches of Dewar and McKendrick, as well as by other investigators, while Chodat[2] has shown that the spores of *Mucor mucedo* survive exposure to from $-70°$ C. to $-110°$ C. for two hours[3]. Similarly many Palmellaceae, Cyanophyceae, &c., can withstand prolonged desiccation over sulphuric acid, and, as has already been shown, complete freezing also, even when in the vegetative condition. Such are *Haematococcus pluvialis*, *Pleurococcus vulgaris*, *Pleurococcus miniatus*, *Rhaphidium polymorphum*, *Glococapsa*, *Merispomedia*, *Nostoc commune*, *Oscillaria antliaria*, *O. tenuis* and *O. subfusca*. On the other hand, Confervae, *Haematococcus nivalis*, the zoospores of

[1] See Assim. Inhib., l. c., p. 402.

[2] Chodat, Bulletin Herb. Boissier, 1896, p. 870.

[3] Brown and Escombe subjected a variety of air-dried seeds to a temperature of $-183°$ C. to $-192°$ C. for 110 hours, and found that their germinative power was not perceptibly affected. Proc. Roy. Soc., lxii, 1897, p. 160.)

Protococcus pluvialis, *Oscillaria sancta*, and vegetative Diatoms and Desmids are killed by air-drying and also by being completely frozen. The zygospores of *Zygnema* can, however, withstand several years' air-drying, and are therefore hardly likely to be affected by cold [1]. The filaments of *Vaucheria* and the normal vegetative mycelia of *Penicillium*, *Mucor*, and *Physomyces* are killed by drying and also by being completely frozen at −10° C. to −12° C. In *Mucor* and *Penicillium* the mycelia may be made more resistant by growing them on concentrated nutrient solutions, while in the form of Mucor yeast, the resistant powers are very different from those of the normal vegetative Mucor mycelium [2].

With regard to marine Algae, many of these are, as the researches of Kjellmann upon the Arctic Flora have shown, very resistant to cold, and it is possible, and indeed probable, that as a class they are more resistant to cold than those fresh-water Algae are, which are purely aquatic. According to Pfeffer [3], in sea-weeds the internal isosmotic force which the cell-sap exerts must be greater than in land-plants, and also than in fresh-water plants, for the former are frequently subjected to great external pressure and are also surrounded by a weak saline solution, and yet the phenomena of turgidity which they present are in general similar to those exhibited by land or fresh-water plants. Hence, in seaweeds the cell-sap will be more highly isosmotic, or more highly concentrated than in fresh-water plants, and therefore a lower temperature will be required to completely freeze the former than the latter. Similarly, just as in *Penicillium*, where the adaptation to growth in concentrated solutions causes an increased

[1] See Schröder, Bot. Unters. Tübingen, Bd. i, Heft 1, 1886; Ewart, Assim. Inhib., 1895, l. c., p. 375, &c.

[2] Chodat, l. c., supposes that spores of *Mucor mucedo* which had commenced to germinate might nevertheless resist exposure to −70° C., but the details of his experiments do not support this conclusion, and it is certain from my own observations that the young vegetative mycelium of *Mucor* is killed by several hours' exposure at a temperature of from −10 to −12° C., although if any acrospores or endospores have been formed, these will germinate and produce new mycelia when restored to a normal temperature.

[3] Pflanzenphysiologie, 2. Aufl., Bd. i, sec. 24.

resistant power to freezing and dessication to be developed, so also may the accommodation to a marine habitat induce a correspondingly greater resistant power in plants which have been gradually transferred from the one medium to the other. Kjellmann has indeed observed that arctic marine Algae may continue to grow and even flourish at or even below zero centigrade, a power which no fresh-water Alga is known to possess. We have apparently here to do with an adaptive modification of similar character, but taking place in the reverse direction to that occurring in those Oscillatorias which can grow and flourish in hot springs.

Similarly with regard to those plants which constitute the Flora of the regions of perpetual snow, we find that these are plants which exhibit special adaptive modifications suiting them to their peculiar habitat. It must be remembered that in such regions the snow is commonly extremely hard and compact, and that the surface is usually more or less dirty and covered with dust fallen from the air, along with numerous micro-organisms which find here conditions suitable for their development. When exposed to direct sunlight, the insolation-temperature will be relatively extremely high for all absorbent bodies which are not cooled by radiation, conduction, or evaporation, whereas at night the temperature falls many degrees below zero. Since the snow reflects so much light and absorbs so little, it follows that coloured microscopic organisms may be at a temperature many degrees above zero when exposed to sunlight, although the snow on which they lie shows no immediate signs of melting. Even in gullies or hollows, sheltered from the sun, the amount of radiated heat which reaches the organisms may suffice to keep them unfrozen during the day, although the snow always remains perfectly hard. When the sun is shining upon them, alpine plants are exposed to extremely intense illumination, such as many plants are unable to withstand. Hence a protective red pigment is often found in the microscopical snow-organisms which contain chlorophyll. This pigment absorbs as much as possible of those rays which exercise an injurious

effect upon the chloroplastids and also upon the cell-plasma, and which are least useful in CO_2-assimilation [1].

The plants which compose a snow-flora must be able to withstand sudden and marked extremes of temperature. They must be able to have all or almost all of the water which they contain removed or converted into solid form without being killed, and must be capable of an almost immediate resumption of both respiration and assimilation when the water is restored, either directly when the plant is dry or by thawing when it is frozen. If any prolonged latent period of recovery always followed as a necessary consequence after short but severe exposure, the plant would waste most of the precious day-time exposure in recovering and preparing for the full resumption of all its vital activities when returned to more favourable conditions. It has already been shown that exceedingly resistant plants do actually exist, which even after prolonged air drying or exposure to severe cold remain living, and almost instantaneously recommence to assimilate and respire, when the temperature is raised to the optimum, and the normal water percentage restored [2].

Lagerheim found that the flora of the perpetual snow region of Pichincha was composed of four species of *Chlamydomonas* (Volvocineae), *Gloeocapsa*, *Nostoc*, *Navicula*, *Glococystis*, *Rhaphidonema nivale* (Ulothrix form), and also a saprophytic fungus, *Selenotila nivalis*, in all twenty-one species. In Vallidal, Swedish Lapland, Wittrock found about nine species of Desmids. Altogether the known snow-flora comprises about seventy species [3]. Wittrock (l. c., pp. 86, 120) finds that the zygospores of the snow Volvocineae (*Chlamydomonas*) and also the resting-stages (non-motile vegetative condition) can withstand air drying. The same has been shown to be

[1] Ann. of Bot. Vol. xi, 1897, Sept., p. 477.

[2] Assim. Inhib., l. c., pp. 385, 389. (Mosses, Lichens, Protophyta.)

[3] Lagerheim, Die Schneeflora des Pichincha, Ber. d. Bot. Gesell., 1892, Bd. x, p. 517; V. B. Wittrock, Om Snöns och isens flora, särskeldt i. de arktiska traktema. Stockholm, 1883.

the case in *Gloeocapsa*, *Nostoc*, *Glococystis*, &c. These plants can therefore also resist great extremes of cold. *Navicula* and other Diatoms do not appear when in the vegetative condition to be so resistant, and actively vegetating Desmids are still less resistant. As regards *Selenotila* and *Rhaphidonema*, no data are at present available.

The observations recorded by Messrs. West (l. c., p. 35) that plants of *Closterium striolatum* and *Cylindrocystis Brebissonii* were found growing in the water derived from melting snow at a (probable) temperature of 1° C. to 2° C. is interesting but not surprising, and has moreover but little scientific value, since the actual temperature of the water was not determined.

With regard to the effects of direct sunlight upon fresh-water Algae, Messrs. West have apparently here fallen into the error of supposing that because the surface of a shallow pond is exposed to direct sunlight, that therefore the plants growing in it are exposed to the same intensity of sunlight as well (l. c., pp. 35 and 36). It is perhaps hardly necessary to mention the fact that unless the sun is directly overhead, a larger or smaller percentage of the incident rays fail to penetrate the water, which is an optically denser medium, and at the surface of which both refraction and reflection take place. As is well known, when the incident angle is an acute one, a very large part of the light, or almost the whole of it, may be reflected without penetrating below the surface; and since the shadow of a landscape upon a still surface of water may appear on a photographic plate to have almost the same intensity as the original picture, it follows that the more refrangible rays with which we are immediately concerned must be reflected in as great if not greater amount than the less refrangible rays are. The oblique light-rays which actually penetrate are bent towards the perpendicular, and hence traverse a shorter distance to reach the bottom of a pond. This may be of considerable importance in the sea by enabling oblique light-rays to penetrate to a greater depth, and thus allow the marine

flora to extend into deeper water than it otherwise could; but in a shallow exposed pond it may occasionally cause a dangerously intense perpendicular illumination to be still further prolonged. Moreover, ordinary pond-water teeming with living and dead particles is only semi-transparent, while, as has in various places been shown[1], an apparently trifling protection—such as a sheet of oiled tissue-paper, or the inclination of the leaf obliquely or parallel to the incident rays, afford—may suffice to adequately shield the sensitive tissues or organs from any injurious effects which prolonged and continuous exposure to direct sunlight might have produced.

When a mass of green Algae has been floating on the surface of a pond exposed to direct sunlight, frequently the uppermost filaments may be entirely or largely bleached, and either still living or dead[2]. Whether this takes place or not, the filaments beneath will always be subjected to a very much diminished intensity of illumination. There can hardly be any doubt but that in an ordinary pond, even when comparatively shallow, the brighter the sunlight, and the more there is of it, the more active will be the growth of the vegetation in the pond as a whole. This is, however, simply because then the average amount of light which the vegetation as a whole receives, approaches nearer to, but does not surpass, the optimal intensity for continued assimilation. The very air or oxygen bubbles, with which an assimilating filament may become covered, cut off from it a certain amount of the light which would otherwise reach it. For all these reasons, when the illumination is comparatively weak, green organisms can develop on the surface only.

When, however, exposure to bright light induces a formation

[1] Assim. Inhib., l. c., 1895 and 1896; Ann. of Bot. Vol. xi, Sept., 1897.

[2] Partially bleached filaments of *Spirogyra* may remain living for a considerable time, and may show a feeble evolution of oxygen from the entire chlorophyll band or from parts of it only. If the bleaching is at all marked, a regeneration of chlorophyll is possible only in the young cells.

of zygospores (West, l. c., p. 36), it does so probably because it is acting injuriously upon the vegetative filaments, for conjugation appears to be induced in Conjugatae, when the external or internal conditions are becoming unfavourable in some way or other. That conjugation might exercise an after stimulating effect upon neighbouring non-conjugating cells, arousing them to increased vegetative activity, is not impossible, provided that plasmatic connexions existed, or that a plasmatic transference were proved to take place through the partition-walls of neighbouring cells of the same filament. This has not yet, however, been demonstrated in these plants, and the occurrence of lateral conjugation by special conjugating tubes connecting neighbouring cells of the same filaments renders the existence of any such inter-protoplasmic communication extremely improbable. Physiologically, each adult cell of a *Spirogyra*-filament appears to be a distinct individual. The fact that when conjugation takes place, the activity of the neighbouring cells appears also to be increased, may simply indicate that the same causes which are inducing conjugation in certain cells are stimulating others to an increased vital activity manifested in other ways. Recent research has shown that injuries and injurious agencies generally, if not too severe, may cause an increased respiration, an increased production of heat, a commencement or increase in the rapidity of rotation, and generally may cause a more marked and active conversion of potential energy into kinetic, while upon the primary processes of constructive metabolism, at any rate as regards CO_2-assimilation, the reverse effect is produced[1]. In many cases the energy liberated is partially expressed in the form of an increased growth activity, precise determinations of which have been given by Townsend and the writer in certain special cases[2]. An increased katabolism, however, involves

[1] Richards, Ann of Bot, Vol. x, 1896, p. 531, Vol. xi, March, 1897, p. 29; Ewart, Ann. of Bot., Vol. xi, Sept., 1897, p. 447, &c.

[2] Townsend, Ann. of Bot., 1897, p. 509; Ewart, Ann. du Jard. bot. de Buitenzorg. 1898, Vol. xv, p. 198, &c.

a greater production of waste katabolic products, some of which (organic acids and their salts) are highly osmotically active. An increased turgidity is therefore to be expected in such cells, other factors remaining constant, and provided that the substances used up in katabolism are either replaced, or are not at all or only slightly osmotically active. It is perhaps in this way and by the softening of the cell-wall at certain points, that the fertilization-tubes are produced in *Spirogyra*. The subsequent contraction of the protoplasts before actual conjugation takes place might be either an active or a passive one, the water filtering out under marked pressure in the first case or slight in the second. If the contraction of the stretched protoplast is a passive one, then either the plasmatic membrane must have become more permeable, or else the osmotic concentration of the inclosed cell-sap must have undergone a rapid and marked diminution. That the latter is possible and even probable is shown by the fact that in the formation of the zygote a marked conversion of osmotic substances into non-osmotic ones takes place (sugars, &c., into oil, starch, &c.). In this way a rapid lowering of the internal osmotic pressure might be produced previously to conjugation. In any case the result is the same, a very resistant body, the zygote, being finally produced, which is very well adapted to withstand desiccation, extreme cold, &c.

From all the facts mentioned above, it is easy to see that only by means of laboratory investigations, in which the experiments are performed under otherwise constant conditions, can any precise conclusions be arrived at with regard to complicated vital phenomena, nor is any determination of the resistant powers of a plant to any given agency possible, unless all other external agencies or modifying factors are allowed for, removed, or kept constant.

That intense illumination acts injuriously upon all exposed living parts of plants is now quite certain, as may be seen by reference to various sources, commencing with Pringsheim's

classical researches, and ending with the observations made by the writer[1].

The same injurious action of intense light is produced upon all plants, whether with or without chlorophyll, though in chlorophyllous cells it is almost always the chlorophyll-grains which are first affected[2]. Thus both non-chromogenic and chromogenic Bacteria, including those which contain chlorophyll and grow normally only when exposed to diffused daylight, may be killed by prolonged exposure to direct sunlight, and even the vitality of the spores may be fatally affected by somewhat more prolonged exposure[3]. It is for this reason that the spores of Mosses, Ferns, &c., and the pollen-grains of higher plants, are covered by comparatively opaque external coverings, for in order that the full effect of the sunlight may be produced, it must penetrate with undiminished intensity to the living contents of the cell.

It is extremely improbable that any living protoplast if naked, or if enclosed by a transparent wall, could withstand exposure to the full and undiminished intensity of sunlight for even a day (twelve hours' constant exposure), and certainly not when exposed successively day after day. In a chlorophyllous cell it is the chlorophyll-grains which are first affected. The reason for this is that these are organs specially adapted for collecting and intercepting sunlight. Light-rays, which pass unaltered directly through the cell, cannot possibly exert any effect upon it. It is only the rays which are altered in their passage through, or actually absorbed, which can exert any influence upon the living plasma or plasmatic plastids. Hence it is upon the chlorophyll-bodies that the most marked effect is produced by intense illumination[4]. It is important to remember in this

[1] Assim. Inhib., Journ. Linn. Soc., Vol. xxxi, pp. 439, 443, 573; Ann. of Bot., Sept., 1897, Vol. xi, p. 339; Pringsheim, Pringsh. Jahrb., Bd. xii, 1882, pp. 326, 345; the literature quoted in these works.

[2] Journ. Linn. Soc., Vol. xxxi, p. 573 (Chara); Ann. of Bot., l. c.

[3] See Frankland and Ward, Report of Thames Water Commission; Ward, Annals of Botany, March, 1898, Vol. xii (A Violet Bacillus), p. 65.

[4] Assim. Inhib., Journ. Linn. Soc, Vol. xxxi, 1896, p. 573, &c.

connexion that a substance transparent to the eye may be opaque to particular rays, and that a colourless tissue may reflect or absorb a considerable portion of the light falling upon it.

The effect produced upon the chloroplastids is not, however, directly proportional to the total amount of light which they absorb, for it is the blue-violet rays which produce the greatest photo-chemical effect, whereas the chlorophyllous absorption in this region of the spectrum is not nearly as great as it is in the red.

The following experiments illustrate these points more clearly, and give more precise data concerning the duration as well as the intensity of the exposure which chloroplastids can successfully withstand. Perfectly accurate data could be obtained only by experimenting with a constant source of illumination, the intensity of which could be varied at will, for when sunlight is used its intensity may continually alter during the exposure. Hence in the following experiments the sunlight was concentrated and the exposure correspondingly shortened, while by using single cells or unilamellar cell-aggregates the intensity of light which reaches the chloroplastids is but little diminished. End-cells of *Chara foetida* and the laminar portions of the leaves of *Elodea* were therefore exposed in water to the sun's rays, concentrated by means of an ordinary concave mirror, and previously cooled by passing through a cold solution of alum.

The image of the sun thrown at the level of the microscope stage was rather less than $\frac{1}{80}$th of the area of the reflecting mirror, but the photo-chemical intensity (for silver salts) of the concentrated sunlight was much less than it should theoretically have been, namely 25 to 30 times greater than that of the direct sunlight, or after passing through an alum solution only 8 to 10 times greater. The intensity of the light employed is indicated by the terms 4 S, 6 S, 8 S, &c., where S represents the full intensity of the strongest sunlight.

Elodea canadensis, leaf-cells exposed in water, cooled by evaporation and with plenty of oxygen.

1. 5 minutes' exposure to about 6 S; chloroplastids pale greenish yellow, rotation absent or very slow, and in 10 min. more rapid, but occasional cells die and rotation ceases. After 1 hour tested with bacteria, weak power of assimilation, absent or doubtful in parts[1].

2. Cell showing one rotation per 25 sec. after $2\frac{1}{2}$ min. in 5 S, rotation slows to 55–60 sec. per revolution 2–3 min. after exposure; then in 5 min. begins to quicken, and in 10 min.= 35 sec. per revolution, in 20 sec. original rate. Chloroplastids slightly paler.

3. No alum-solution, 1 min. exposure stops active rotation, chloroplastids quite green. After 1 hour still no rotation, but apparently a weak evolution of oxygen; but careful examination shows that the movement of the bacteria continues in the darkness, and that many semi-anacrobic bacteria are present, and are attracted by the nutritious fluids evolved from the dying cells. Using bacteria from a young and well-aerated culture, no evolution of oxygen can be detected, and after 5 hours cells are no longer plasmolysable and replasmolysable.

4. (*a*) With alum-solution, light = 4 S; after 15 min. exposure chloroplastids partially bleached, feeble rotation, which become active after $\frac{1}{4}$ hour in weak light.

(*b*) Light = 4 to 6 S; after 15 min. rotation almost ceased, begins to recover in 5 min., and is quite active again in 15. Chloroplastids more or less bleached.

(*c*) Closed-cell-preparation. Owing to increased heating effect rotation ceases in nearly all cells after 5 min., recovering again in 5–15 min. if only a part of the leaf was exposed. After 10 min. exposure, permanent stoppage, cells die, but the chloroplastids are not bleached (deficient supply of oxygen).

5. (*a*) Light = 8 S, rotation ceases in 5–6 min., chloroplastids quite pale, and in 6–8 min. quite colourless, no recovery. After 5 min. exposure the cells mostly recover, rotation recommences in $\frac{1}{4}$ to 1 hour, and after being in diffuse light for 2–3 days paler exposed patch can no longer be distinguished from rest of leaf.

(*b*) Without alum-solution, rotation ceases in 3–4 min., chloroplastids still yellowish green, recovery in many cases but not in all.

[1] Longitudinal strips are most suitable for examination. The Bacteria were obtained from well aerated putrescent fluids (See Kny, Ber. d. D. Bot. Ges. xv, 1897, p. 388), but the utmost caution is necessary with such impure cultures, and were it not that the results here given are simply confirmatory of previous ones, no mention would be made of them.

In *Chara foetida* about 10 minutes' exposure to cooled and concentrated light (8 to 10 S) is sufficient to completely bleach a naked and non-encrusted end-cell. The chloroplastids on the under surface bleach first, those on the upper at the same time or immediately afterwards, and those at the sides a minute or two later. If the exposure and bleaching are localized, the cell may remain living, and the majority of the bleached chloroplastids retain their original position, so that, although a few come free and are carried away by the rotating plasma, no naked areas are formed, as was the case in Pringsheim's experiments[1]. The rotating plasma experiences a certain check at the exposed area, and finally the current may cross the cell at this point and return up the other side, the endoplasm being thus divided into two distinct rotating masses. Such cells always die when returned to normal conditions. By suddenly exposing preparations of end-cells of *Chara* to light, it is easy to show that the bleached portions have no power of evolving oxygen by CO_2-assimilation; and when such end-cells are kept in 2% glycerine, starch-grains are formed in the green chloroplastids, but not in the colourless ones, which have therefore not only lost the power of CO_2-assimilation, but also of starch formation.

Similar results were obtained with leaf-cells of *Elodea*, and although it has not been found possible to entirely bleach all the chloroplastids of a cell without killing the latter, still living cells may be obtained in which a portion of the chloroplastids are entirely bleached, and the rest are pale yellow to yellowish green. The latter may become normally green again, and are able to form starch, whereas however long the leaves may be kept in a 5% sugar-solution, no starch-grains appear in the bleached chloroplastids. Living end-cells of *Chara* have been observed in which the bleached chloroplastids were retained for an entire year without undergoing any further change[2], and hence apparently light not only kills and bleaches the chloroplastids, but also converts its proteid

[1] Pringsheim, Pringsh. Jahrb., Bd. xii, 1882, pp. 326 344.
[2] Journ. Linn. Soc., 1897, Vol. xxxi. p. 573.

substance into some extremely insoluble modification. It is possible to completely bleach all the chloroplastids of a leaf-cell of *Elodea* without previously active rotation entirely ceasing, and when returned to normal conditions rotation may become active again in a quarter to half-an-hour. All such cells die in water in a day or two, though they may be kept for as long as a week in dilute sugar or glycerine.

A few experiments were also made upon a couple of chlorophyllous animals, *Hydra viridis*, with definite 'chloroplastids,' and *Vorticella campanula*, with diffuse chlorophyll.

Vorticella campanula. 1. Light = 4–5 S, killed in 1 min. in closed cell preparation without any perceptible bleaching of the chlorophyll. In an open drop of water the animals are killed (no alum-solution) in 3–4 min., but behind an alum-solution 5–6 min. are required, and in this time the chlorophyll is almost completely bleached. The contractions and expansions take place more slowly in 2–3 min., and finally the stalk may remain contracted, and the body contracted or expanded in light rigor. Partial recovery may take place, a portion of the cilia commencing to move again. A weaker intensity of light may at first accelerate movement, the contractions and re-expansions of the stalk taking place at shorter intervals.

2. Secondary effect of light. After exposure to the brightest diffuse daylight for 1 day, almost all the *Vorticella* zooids separate from their stalks and become free swimming. This is probably an attempt to escape an unfavourable intensity of light.

3. 8–10 S, movement almost immediately ceases, recommencing in 1–5 min., according to the length of the (non-fatal) exposure. Temporary light-rigor if suddenly induced may leave the stalks and bodies more or less completely expanded, and on removing the light the first movement may be to complete the previously commenced coiling. If the light-rigor is at all prolonged (2–3 min.), death immediately follows.

Hydra viridis. 1. Exposed to 6 S, at once retracts, and remains so till death occurs. Ends of tentacles bleached in 10 min., body still dark green after 15 min., but power of recovery is lost. It is possible to permanently destroy the motility of some of the tentacles by localized exposure, while the rest remain living. After 10 min. exposure tentacles may be killed, but the body may show feeble

contractions and expansions ½ hour afterwards, stronger after 1 hour, but permanently ceasing after 5 hours.

2. 8–10 S, after 5 min. animals killed, body greenish yellow, tentacles bleached. With shorter exposure temporary light-rigor may be produced in some of the tentacles, and by localized exposure the middle of the animal's body may be killed and contracted, while the base and apex remain living for several hours, and capable of responding to light, &c. Hydrae, in which the body is still greenish and capable of expanding and contracting, may show no perceptible evolution of oxygen when exposed to light.

It appears therefore as if the plasma of a chlorophyllous animal is more sensitive to light than that of a plant such as *Chara*, for the animal is killed before the chlorophyll is bleached, whereas almost the whole of the chloroplastids may be bleached without producing any permanent injury upon the rest of the cell[1]. This is well illustrated in the following comparative experiments, in which the light concentrated, after passing through an alum-solution, had a photo-chemical intensity ten times greater than that of the strongest direct sunlight.

Elodea canadensis. After 5 min. all exposed cells bleached and killed. If the end of a cell is exposed, only the chloroplastids stationary here bleach, and such cells may show rotation extending up to the bleached part but not into it, while cells at the margin of the exposed area with green chloroplastids may show variations and changes in the direction of the rotation, or the chlorophyll-grains may be aggregated into a rotating ball at one end of the cell, or rotation may even be shown in cells with partially retracted contents.

Chara foetida. Complete bleaching requires nearly 8 min. exposure. After ½ min. rotation may be twice as rapid, but in 4–5 min. slows rapidly, is very slow by the time the chloroplastids are bleached, and may gradually stop after the exposure has ceased, but in other cases may recover again. When the entire cell is exposed, complete bleaching almost always involves the rapid or immediate death of the cell.

[1] The bleaching of the chloroplastids is an oxidatory process, and less oxygen may reach the chlorophyll in an animal's body than in a plant-cell.

Hydra viridis. The animals instantly retract, and after 3–4 min. projecting parts of tentacles slightly bleached, body quite dark green. Recovery is possible, and after ½ hour partial expansions and contractions, more active after 1 hour. After 5–6 min. exposure the animals are killed, but hardly at all bleached.

Vorticella campanula. 3–4 min. causes complete bleaching, but the animals may be fatally affected a minute sooner than this.

Streptococcus varians[1]. Thick turbid culture of this green bacterium was entirely decolourized by 5 hours' exposure to direct sunlight, though the bacteria reappeared again later.

The independence of rotation and CO_2-assimilation, and the fact that assimilatory inhibition is not necessarily dependent upon the destruction of the chlorophyll, have already been established. Chloroplastids of *Chara* rotating in the endoplasm are shielded from the light by the outer chloroplastids, and may remain green after the exoplasmic layer has been completely bleached. In *Elodea*, however, the chloroplastids all assume the apostrophic position, and rotating corpuscles are exposed to the same or an even greater intensity of light than outer stationary ones; but nevertheless the former are less rapidly affected than the latter, probably because the exposure is more evenly distributed as the rotating chlorophyll-corpuscle turns over from time to time. As the rotation slows, the chloroplastids may distribute themselves to a greater or less extent over the cell, and both these factors aid somewhat in producing more rapid bleaching. In all cases the bleaching apparently takes place with markedly increasing rapidity towards the end of the exposure as the chloroplastids become fatally affected. Thus with an intensity of light sufficient to completely bleach the chloroplastids in six minutes, during the first four minutes but little change in colour may be apparent, while during the fifth minute the corpuscle becomes much paler green, and during the sixth minute all green colour is lost. If we assume that the decomposition and reconstruction of chloro-

[1] Journ. Linn. Soc., Vol. xxxiii, 1897, p. 150.

phyll proceed simultaneously in every chloroplastid exposed to light, and that the former process is more rapid than the latter when the light is strong, then the apparently more rapid bleaching at the end of the exposure might be due to the reformation of chlorophyll having ceased to take place, and this would indicate that fresh chlorophyll can normally be produced with great rapidity, though not much more rapidly than the chloroplastids of *Spirogyra* can form starch[1]. It is, however, extremely difficult to estimate changes of coloration by comparison with the surrounding chloroplastids so long as the exposed ones are distinctly green, and the same loss of chlorophyll which converts a pale green chloroplastid into a colourless one may cause no perceptible difference in the coloration of a dark green one. As a matter of fact, the leaves of many plants may yield a much weaker extract of chlorophyll after a day's exposure to strong light than is obtained from a similar bulk of shaded leaves, although the eye may be unable to detect any difference in the depth of coloration of the two sets of leaves, and hence it is doubtful whether the apparently more rapid decomposition of chlorophyll as the chloroplastids become fatally affected is not simply an optical delusion.

Assuming that this peculiarity does actually exist and cannot be entirely explained by the facts already given, it might be possible that the chlorophyll, when in organic connexion with a living plastid, is more resistant to the oxidatory photo-chemical action of sunlight than it is when isolated, or when the organic union is disturbed or destroyed. If plants of *Elodea* are killed by chloroform, and exposed in well-aerated water to the action of bright light for a day, including nearly three hours' intermittent sunlight, all the exposed leaves are completely bleached, whereas a normal healthy plant subjected to the same exposure retains a healthy green colour, though perhaps slightly paler.

[1] G. Kraus, Jahrb. f. Wiss. Bot., 1869–70, Bd. vii, p. 511.

Similarly living plants remain a normal green in bright diffuse daylight, whereas dead ones are bleached in a couple of days in the presence of oxygen, although if placed in well-boiled water, covered by a layer of oil, they retain their green colour for an almost indefinite length of time. Unless we admit that the decomposition and the reformation of chlorophyll proceed simultaneously when the living chloroplastid is exposed to light, the above facts can only be explained by assuming that in the living chloroplastid the chlorophyll is held in some such manner as to render it resistant to the action of light, and that it is only when released from this vital combination that it can be oxidized and decomposed.

Chlorophyllous cells, however, suddenly killed by chloroform, and exposed to intense light as soon as the chloroform has evaporated, take from 2—3 times as long to bleach as living ones do. In such dead cells the plasma is slightly more opaque, but on the other hand the slightly bluish green dead chloroplastids are distributed evenly throughout the cell, and are hence exposed to a slightly greater average intensity of light on this account. Living chloroplastids may at first evolve oxygen, and hence help to bleach themselves, but this certainly ceases long before the bleaching is complete. If a closed-cell-preparation of a leaf of *Elodea*, which has been kept in darkness for some time to remove all free oxygen, is suddenly exposed to cooled and 10 times concentrated sunlight, the leaf-cells are killed without any perceptible bleaching of the chlorophyll having taken place, and hence it is evident that in such intense light CO_2-assimilation ceases almost immediately, if any is ever possible. Similarly if a leaf of *Elodea* is passed through chloroform, a condition of anaesthesia may be induced which lasts for five or ten minutes, or even longer. Rotation ceases, the chloroplastids distribute themselves regularly over the cell, the power of response to external stimuli is temporarily in abeyance, and from the results given later it is also certain that the power of CO_2-assimilation is temporarily lost. Such

cells take only a minute or two longer to bleach than normal living ones do. Hence it appears that the difference is due to the fact that in the dead cell light exercises a photochemical action upon a dead inert substance, whereas in the living cell it acts upon a working vital mechanism composed of substances which are continually undergoing change, and causes katabolic and oxidatory changes to preponderate over the anabolic ones. It must be remembered that there is no necessary connexion between the bleaching of the chlorophyll and the death of the chloroplastid, and it may be found possible, under certain circumstances, to completely bleach chloroplastids without killing them. As a matter of fact, cases have been observed in *Elodea* in which the chloroplastids were almost entirely bleached, but yet remained living and capable of recovery.

Even assuming that the rate at which the chlorophyll is decomposed in the presence of oxygen is directly proportionate to the intensity of the light, it does not necessarily follow that in unconcentrated sunlight decomposition is also more active in the living chloroplastids than it is in dead ones. A living end-cell of *Chara* is, however, bleached by 6–8 hours' exposure to continuous sunlight (6–7 minutes when exposed to sunlight 10 times concentrated), whereas an end-cell killed by chloroform is bleached under similar conditions in about 2 hours, but when exposed to 10 times concentrated sunlight 12–15 minutes' exposure is required. It seems therefore justifiable to conclude that during the period of exposure to direct sunlight the living chloroplastids had formed at least five times as much chlorophyll as they originally contained. Without attaching too much importance to these calculations, they are nevertheless interesting as showing the perpetual change to which the living chloroplastid is subject when exposed to light. Under prolonged exposure to the same intensity of sunlight, old chlorophyllous cells bleach much more rapidly than young but fully grown cells do; whereas in concentrated sunlight, in which the bleaching is produced in a few minutes, no marked

difference is perceptible. This is evidently due to the fact that the reconstruction of chlorophyll is more active in the younger chloroplastids, and it is easy to see why chlorophyllous organs, when the sunlight is discontinuous, may be able to withstand a far greater total exposure than when it is continuous.

It is of considerable interest to know whether there is a certain maximal intensity of light beyond which CO_2-assimilation immediately ceases to be possible, so that momentary exposure to such light acts just as momentary darkness does, causing assimilation to cease until normal conditions of illumination are restored. Intense illumination, however, soon puts the assimilatory mechanism out of order, and hence a direct stoppage of CO_2-assimilation is possible without the chloroplastids being markedly affected, only when the exposure is extremely brief. The following experiments were all performed with cooled and 10 times concentrated sunlight. The bacterium-method is not directly applicable, for any Bacteria which come within the circle of intense light are at once rendered immotile, and moreover a closed-cell-preparation must be employed. After 5 minutes' exposure in a drop of water the power of assimilation is lost by the exposed parts of an *Elodea* leaf, and may either not return, or, if the chloroplastids are still green, recovery may take place in one hour to a day. In a closed-cell-preparation, rotation may be stopped and may never recommence if the entire leaf has been exposed, the leaf-cells dying of asphyxia in 2–3 hours, whereas if oxygen is admitted, rotation recommences and the cells may remain living. The chloroplastids have therefore temporarily or permanently lost the power of producing oxygen; for in a normal closed-cell-preparation exposed to light of moderate intensity, the oxygen evolved by CO_2-assimilation suffices to maintain active rotation, the latter ceasing after 10–15 minutes' darkness, and recommencing within a minute in the light, though it does not become fully active until several minutes have elapsed.

If a leaf of *Elodea* is exposed to light in water containing carbonic acid, minute bubbles of gas ooze out from the ends of the interspaces in the leaf[1], but when exposed to concentrated light, this ceases almost at once, and instead water may be sucked in, either owing to the cessation of CO_2-assimilation, or to the increased oxidatory activity consuming all the oxygen that would otherwise have been set free. Reinke[2] found that *Elodea* gave off gas-bubbles most actively in direct sunlight, the activity being unchanged even when the light was 60 times concentrated, and the evolution of gas-bubbles diminished only when the plants were injuriously affected by a much greater intensity of light than this. The intensity of the light falling upon the outside of a glass vessel is, however, much greater than that which penetrates the living cells, and the optimal and maximal light-intensities for CO_2-assimilation are not those which fall upon the outside of the plant, but those which reach and react upon the *chloroplastids*. There is good reason to suppose that in all cases the optimal light-intensity for *continued* CO_2-assimilation in the chloroplastid is less than that of direct sunlight. When plants are exposed to intense sunlight in water, the rapid rise in temperature causes the intercellular air to expand, and may appreciably affect the rate at which gas-bubbles are evolved; and it is possible, by rapidly warming the water in which recently killed plants lie, to produce a slight evolution of bubbles from the cut end of the stem, just as if feeble CO_2-assimilation had taken place[3]. Hence it is doubtful whether the evolution of gas-bubbles noticed by Reinke from plants exposed to 60 times concentrated sunlight was actually due to any CO_2-assimilation taking place at this intensity of illumination.

It appears therefore that in living chloroplastids exposed to light, the decomposition and reconstruction of chlorophyll proceed simultaneously, and that in certain cases the total

[1] Cf. Kohl, Ber. d. Bot. Ges., 1897, Bd. xv, p. 120.

[2] Reinke, Bot. Ztg., 1883, p. 713.

[3] See note on Chloroform anaesthetization, *infra*, p. 415.

amount formed in a single day may be several times greater than that present at any given moment. Light acts both as a stimulus to the formation of chlorophyll, and at the same time induces its photo-chemical oxidation. The former is a vital action, and is by no means directly proportional to the intensity of the illumination, for ordinary diffuse light appears to afford the optimal stimulus to chlorophyll formation. The decomposing action of light is, however, directly proportional to its photo-chemical intensity, if an abundant supply of oxygen be present. Hence in strong light the decomposition of chlorophyll preponderates, and it is easy to understand why an etiolated plant turns green more rapidly in diffuse daylight than in direct sunlight. The inhibitory effect exercised by strong light upon the assimilatory powers of the chloroplastids is not necessarily the result of a diminution in the amount of chlorophyll which they contain, but may be due to some break having occurred in the chain of processes which constitute CO_2-assimilation, or perhaps to the necessary vital connexion between the plasma and the newly-formed chlorophyll not being properly established. It is, however, inadvisable to push these theoretical conclusions too far, nor do these experiments afford any support to Pringsheim's ingenious hypothesis as to the protective function of chlorophyll.

The purpose of my own experiments has throughout been to determine how long the living chlorophyll-grains in the intact cell can withstand exposure to direct sunlight, and what length of time elapses before their functional activity is perceptibly affected. Hence when compound tissues, composed of several layers and covered by an epidermis, were employed, the light was frequently concentrated by mirrors, or with more sensitive leaves these were simply kept exposed to perpendicular illumination for prolonged periods; while when water-plants, &c., were employed, care was taken that the full intensity of the illumination actually reached the living contents of the cells.

Once the intensity of the illumination has been deter-

mined, beyond which any increase in the light reaching the living cells injuriously affects them, it is possible to calculate the intensity of the illumination to which the plant as a whole may in nature be exposed without any particular cells forming part of it being directly injured, provided the amount lost by the absorption and reflection of the interposed protecting layers, &c., is known. For an account of the adaptive modifications shown by the tropical plants, and the means by which those growing in exposed situations are able to protect themselves from the effects of over-exposure, reference may be made to the paper on the effects of tropical insolation[1].

Here, except where otherwise mentioned, the thermal and photo-chemical effects were allowed to act together, but even then it was found that the leaves of most of the plants examined were resistant to even prolonged exposure to continuous direct sunlight, although slight injurious effects might be produced upon them. Naturally, if but one surface is directly exposed, only the cells immediately beneath this surface become perceptibly affected. By using a mirror, however, the under surface of the leaf may be illuminated as well, and the cells in the interior subjected to an intensity of illumination approaching more closely to that of direct sunlight. Many shade-plants are comparatively sensitive to prolonged exposure to direct sunlight. Thus the chlorophyll-grains of *Pisonia alba*, *Selaginella sp.* (?), *Chara hispida*, *Spirogyra crassa*, &c., may be almost or entirely bleached by a single day's exposure, and although they have temporarily lost the power of assimilation, on returning to normal conditions they may become green and commence to assimilate again in a few days (l. c., pp. 442, 443). Trans-

[1] Ann. of Bot., Sept., 1897, Vol. xi, p. 339. Giltay (Annales du Jardin Bot. de Buitenzorg, 1898, Vol. xv, p. 68) has shown that *Cassia timorensis* in diffuse daylight assimilates 6·4 mg. CO_2 per ½ sq. m. of leaf, but when exposed to strong sunlight only 5·7 mg. CO_2, owing to the erect position which the leaflets assume, and hence owing to this protective adaptation the optimal intensity of illumination for the assimilation of the plant as a whole is markedly reduced.

piration keeps thin exposed leaves cool so long as an adequate supply of water is available, but in many fleshy leaves this is not the case, and the effect produced upon the power of CO_2-assimilation may be largely a thermal one, for the temperature of such leaves, when isolated, may amount to as much as 50° C. (l. c., p. 444). When at the same time any discoloration is produced in the chlorophyll-bodies, the leaves, instead of being blanched, turn brown or yellow (l. c., *Vanilla*, *Hoya*, *Cocculus Beccarii*), and it is curious that the action of light, combined with low temperatures, may cause a similar browning[1]. Thus at ordinary temperatures the photo-chemical action of light causes a blanching of the chloroplastid, whereas at extremes of temperature a browning may be produced, if the leaves remain living for a sufficient length of time under such exposure. In the majority of cases, however, healthy and normal leaves are comparatively resistant to prolonged exposure to direct perpendicular sunlight, even when the under surface is also illuminated, no perceptible effect being produced, or only a slight one in the surface layers immediately exposed. Often, however, in such apparently normal leaves, the assimilatory powers may be markedly affected, in parts or entirely. It is important to remember that in a condition of nature, so long as the temperature remains normal, it is very rarely indeed that the exposure is sufficiently intense or prolonged to produce any of the results which may be obtained under the much severer experimental conditions. As soon, however, as precise experimental data are obtained, it is easy to determine the amount of external exposure which any given plant can withstand in a condition of nature, knowing the conditions under which it exists and the protective adaptations which it possesses.

[1] Winter Browning of Conifers, &c., Journ. of Linn. Soc., Vol. xxxi, p. 390.

Botanical Laboratory, Oxford.

On the Development of Arum maculatum from the Seed.

BY

RINA SCOTT AND ETHEL SARGANT.

With Plate XXV.

IN April, 1895, a large patch of *Arum maculatum* was noticed by one of us growing on an open common at Instow, in Devonshire. Most of the plants had but one small ovate-shaped leaf above ground. There were no mature plants anywhere in the neighbourhood.

On digging some of these up, it was clear that they were seedlings, though not very young, but each plant was quite distinct from its fellows. One could trace at least *two* dead tubers of previous years in most cases, though the plants as yet had not produced the characteristic sagittate Arum-leaf.

It seemed worth while to collect some Arum-fruits, in order to study the germination of the seed and generally to test under what conditions the plant is reproduced by seeds and under what by vegetative budding.

Amongst the clumps of mature plants with sagittate leaves a large number of plantlets with ovate leaves are always found. If a clump be dug up, most of these will be found to arise by vegetative budding from the mature tuber (see Fig. 10 A), while a few are true seedlings.

[Annals of Botany, Vol. XII. No. XLVII. September, 1898.]

It was the contrast between the young and the mature forms which suggested the study of the life-history of *Arum maculatum* from the germination of the seed onward.

In July of the same year the mature plants with their spadices of ripe fruits were watched, and on one occasion a pair of chaffinches were seen to clear off the berries from two of these in half an hour. Further evidence of birds eating these berries is much needed, as many country people say that birds do not touch them. However, Gilbert White, in his Natural History of Selborne[1], mentions that the thrush scratches out and eats the tubers of Arums from dry banks, and as the tuber is equally pungent and disagreeable to the taste, there seems no reason why birds should not also eat the berries. The disagreeable taste is principally due to the presence of raphides[2] (see p. 404).

If birds often eat these berries, it will be easy to see how the seeds could have been deposited on the gorse-covered common at a long distance from the parent plants.

Fruits were collected from several ripe spadices, and the seeds sown in flower-pots, as soon as ripe in July. They germinated readily. The earth was disturbed at intervals, and young seedlings in their various stages of germination preserved in spirit.

The growth of the young plants is extraordinarily slow; it is no wonder that the seedling plants have been little noticed, as nothing is to be seen above ground during the first two seasons' growth, and in the third only one ovate leaf shows itself, which at this stage is difficult to distinguish from a leaf of a young plant produced by vegetative budding from the old tubers[3].

The easiest way of distinguishing them is, perhaps, by the length of the underground part of the petiole, which in a

[1] Letter xv.

[2] Stahl, Pflanzen und Schnecken, Jena, 1888, p. 85.

[3] The 'second season' in this paper corresponds to the first season of Rimbach. In England the seeds ripen in July, and germinate in the autumn; whereas in the colder climate of Germany the seeds do not ripen till September, and consequently do not germinate till the spring.

vegetatively produced plant is as long as the distance from the upper part of the tuber to the surface of the ground, and as the tuber of the mature plant is situated from 10–16 cms. deep, the difference in length between this and the seedling petiole is very material.

In a favourable habitat the majority of the new plants are produced vegetatively, for the *Arum* is a plant in which the individual becomes very well established, and is to a great extent independent of reproducing itself by seeds.

It will now be necessary to give a more detailed account of the germination of the seed.

The work in this part of the subject has been necessarily slow and laborious, as the material had to be collected at intervals of a month or less for four seasons.

The seed first swells considerably, then the cotyledon emerges from the seed-coats, carrying with it the plumule and radicle (see Fig. 1). Even at this stage, reached in January, the hypocotyl below the plumule is enlarged to form the tuber, and is packed with food-material.

The stem-bud, which is situated on the tuber and within the hollow cylindrical cotyledon, consists of two leaves, one quite rudimentary, and a growing point.

In the next stage, represented in Fig. 3, April, 1896, both radicle and cotyledon have elongated, and the tuber has doubled its diameter; within the stem-bud a new leaf has appeared, and the bud now contains all the leaves, which will reach maturity during the third season.

In Fig. 4, May, 1896, a rudiment of a fourth leaf, which will not be fully developed until the fourth season, appears.

In Fig. 5, June, 1896, the radicle shrivels and soon entirely disappears. Shortly before this the cotyledon, carrying the seed-coats with it, has been detached from the tuber, leaving the stem-bud exposed. This process is brought about by the formation of periderm [1].

Up to the stage shown in Fig 4 the tuber is covered by

[1] Cf. Parkin, On some points in the Histology of Monocotyledons, Ann. of Bot. June, 1895

a smooth white epidermis. It must be remembered that the insertion of the cotyledon divides the surface of the tuber into two regions. The epidermis covering the lower part is continuous with the outer epidermis of the cotyledon, and ceases abruptly where the primary root begins. The upper part of the tuber is enclosed within the cylindrical cotyledon, and the epidermis which covers it is continuous with that of the inner cotyledonary surface and with the epidermis of the first leaf. The formation of periderm begins in the layer of cells immediately below the epidermis of both regions. Transverse sections through such a tuber as that drawn in Fig. 4 show five or six layers of periderm-cells near the middle of the tuber, and two or three on either side of the zone in which the cotyledon is inserted. These two formations are connected by one or two layers of periderm-cells which cut right across the parenchymatous tissues of the cotyledon, just where it is inserted on the tuber. In older seedlings no doubt a greater number of cell-layers is formed.

The two parts often remain in contact with one another for some time, though organically separated. It is often possible to dig them up without shifting them. In the summer new roots are sent out (from four to six in number) from the upper surface of the tuber (Fig. 6). Some of these, generally two, are contractile[1] (see Fig. 8). We found root-hairs produced in clusters near the tips of the contractile roots at this stage, by which, no doubt, they are firmly fixed in the ground. It is clear that without some point of attachment the effect of the contraction would be to draw the tip of the roots up, rather than to pull down the tuber. But measurements show that the tuber actually sinks deeper into the ground, and in this way is at last freed from its discarded cotyledon and seed-coats, which are left to rot nearer the surface.

The result of the contraction of the rootlets is very startling. The tubers when last examined in May (Fig. 4) were only

[1] Rimbach, Berichte der Deutsch. Bot. Gesell., April, 1897; and Prof. F. W. Oliver, Journal of the Royal Horticultural Soc., April, 1898, p. 493.

about 2 cms. below the surface. On turning up the soil in October for new specimens, none were to be found, and it was not until the soil had been turned up almost 7 cms. that the missing tubers were discovered (Fig. 6). Probably the soil in the flower-pot was looser than it would have been under natural conditions, and this may perhaps have tended to increase the effect of the pull. If a young tuber be re-planted near the surface, it will send out new contractile roots, and in a week will regain its normal depth.

With practice it is quite easy to find seedlings in any stage in nature, even when in the winter resting-condition, by learning the exact depth at which they are to be found at the various times of year.

This is the end of the second season's growth; the whole process has up to this time been carried on underground, and no chlorophyll has been formed.

In the following spring the first ovate leaf, with its two scale-leaves, appears above ground (Fig. 7); the tuber continues to grow in size until June, when next year's tuber is formed.

New roots now arise, some of which are contractile. The leaf withers and the tuber is drawn still deeper into the ground, this time shifting its position as a rule from vertical to horizontal.

It is not, at any rate, until the fourth season, and generally later, that the first sagittate leaves are found. The mature flowering plant (Fig. 10) generally bears three sagittate leaves and two scale-leaves, the inflorescence being enclosed in the sheathing petiole of the innermost leaf (Fig. 9).

It will be seen from this description of the seedling-plant, that a vegetatively produced plant arising, as it generally does, from the lower surface of the mature tuber (Fig. 10 A), in addition to the advantage of procuring its food-stuff ready made, has a further very great advantage over the plant produced from seed, in starting from the first at its normal depth in the ground. In this way it eliminates the risk of being eaten up by thrushes or scratched up by animals, and also saves itself a large amount of unnecessary expenditure of

energy in the yearly production of contractile roots for burying purposes. The result is that the vegetatively produced plant flowers much younger than that produced from seed, which rarely flowers before the seventh year.

INTERNAL MORPHOLOGY.

Any account of the curious second-year seedlings of *Arum maculatum* would be incomplete without some examination of their anatomy. Certain points in their external morphology must otherwise remain obscure. External characters, for instance, are insufficient to determine either the homology of the tuber, or the way in which the cotyledon is detached from it at the proper age (cf. Figs. 4 and 5). Irmisch[1], in describing the mature plant, has rightly stated that its tuber represents the stem-axis of the previous year. This cannot, however, be true of a seedling which has just germinated. The tuber of that drawn in Fig. 2 clearly forms part of the main axis, but we cannot tell from inspection whether the stem alone has been enlarged or part of the root as well. And in that part of the tuber which represents the stem there is no external mark to divide the plumule from the hypocotyl. These questions will of course be settled when the course of the bundles in the tuber has been worked out, but we may so far anticipate our results as to say that the tuber does not show complete root-structure until we reach its base, and it may therefore be considered as belonging entirely to the stem. Moreover, the greater part of it represents the axis of the plumule, for the cotyledonary traces do not bend inwards to join the central cylinder for some distance below the insertion of the cotyledon.

The raphides which give the juice of *Arum* its acrid quality[2] are abundant in cotyledon, stem-leaves, and in the periphery of the tuber. The cells which contain them are larger than the

[1] Irmisch, Zur Morphologie der monokot. Knollen- und Zwiebelgewächse, Berlin, 1850, p. 164.

[2] Stahl, Pflanzen und Schnecken, Jena, 1888, p. 85.

surrounding cells, and are nearly filled by dense masses of needle-shaped crystals. Thick-walled secretory sacs are also found in the tissues of the cotyledon and of the older leaves. Occasionally they even occur in the epidermis. On treatment with salts of iron their contents become black, showing the presence of tannin. We have not found tannin sacs accompanying the vascular bundles.

Before passing on to another part of the subject, we may mention a third point in which the anatomical characters of these seedlings throw light on their external form. The contractile roots of older specimens (Fig. 8) are remarkable for the radial elongation of the cells belonging to the inner cortex. The appearance of the central cylinder in transverse section is in no way distorted by the consequent contraction of the whole root, but the cells of the outer cortex are very much crushed and strained [1]. Sections from the ribbed roots of third or fourth year plants show these peculiarities most plainly.

Hitherto we have dealt only with those points in the anatomy of *Arum* seedlings which serve to complete our knowledge of their external form. For this purpose we have referred briefly to their vascular system. But the detailed study of this system is interesting in itself, and of importance from two points of view, which we will consider separately.

The *course of the bundles* in the mature tuber of *Arum* cannot be deciphered. It is always much more difficult to follow the vascular system in a shortened stem than in one which possesses internodes of moderate length. Two other complications render the task impossible in this case. All the bundles of the central cylinder form a continuous vascular girdle at each node. This peculiarity is found in many genera of the Aroideae [2]. But besides this, all the bundles in the tuber anastomose freely with each other. This is the case even with the leaf-traces which are passing downwards through the cortex to enter the central cylinder at one of the

[1] Rimbach, l. c.

[2] De Bary, Comp. Anat., Eng. ed., pp. 268 269.

lower nodes. Thus a complicated network is formed in which no ground plan can be recognized.

The formation of a vascular girdle at each node is indicated even in the youngest seedlings we possess (Fig. 1). But the bundles which unite these are not completely fused with each other, and can be distinguished throughout their course. The tendency to anastomose is also shown very early. We have found an irregular lateral anastomosis in a seedling no older than that drawn in Fig. 4. No great complications have yet arisen from this cause however, and therefore the course of the leaf-traces can be clearly followed from their entrance into the tuber until they join the central cylinder, as well as the disposition of the bundles in the cylinder itself.

The *transition from stem to root* in the hypocotyl has been described in very few Monocotyledons. In these plants it takes place as a rule with great abruptness, and it is hardly possible to prepare a series of transverse sections from which the course of the bundles in so short a region can be accurately determined, without the aid of a microtome. In his elaborate researches on the anatomy of this region in Vascular Plants, M. Gérard states that his choice of Monocotyledonous species was much limited by the necessity of using those only which possess a moderately long hypocotyl[1]. In fact, he described but nine species, nor has much been done in this direction since the publication of his paper in 1881. The anatomy of the hypocotyl in any Monocotyledon is therefore worth investigation.

Course of the Bundles in the Stem.

At the end of the first year, the three leaves which will come to maturity in the following season are fairly well differentiated in the stem-bud. But to understand the vascular system of the tuber we must choose a seedling in which the cotyledon is still present—such a specimen, for

[1] Gérard, Passage de la Racine à la Tige . . . . Ann. d. Sci. Nat., Sér. 6, Vol. xi, Bot., 1881.

example, as that drawn in Fig. 4. A transverse section passing through the cotyledon and the stem-bud just above the insertion of the latter on the axis, shows the cotyledon as a ring of tissue studded with five vascular bundles. Within it are three concentric rings which represent the three leaves already mentioned. A fourth rudimentary leaf is indeed present, but as it will not come to maturity in the following year, we are not now concerned with its structure.

Each of the two outer leaves displays three bundles, the third shows a single procambial strand representing its midrib. We can trace these seven bundles in a section taken rather lower down, beneath the insertion of the stem-bud but above that of the cotyledon. The position of the bundles in such a section is diagrammatically shown in Fig. 11. The cotyledonary bundles are numbered 1–5, and of these that marked 3 is the midrib. The circles *A*, *B*, *C* represent the traces of the first leaf; α, β, γ, those of the second. The midrib of the third leaf is called *l*. In the node next below this internode, the course of the bundles is indicated by the arrows in the diagram. The inmost circle of traces, α, β, γ, bend inwards, and with the midrib *l*, which forks to the right and left, they form an incomplete vascular girdle. The phloem of the four bundles indeed forms a continuous ring, within which are the groups of xylem. These, though extended tangentially, do not yet unite to form a ring; the xylem of the midrib always remains quite distinct from that of the other bundles. The xylem groups of the latter stretch out in a broken crescent fronting the midrib—an arrangement very characteristic of the young node. It is well shown in Fig. 15 which represents the first node of the young stem.

The node just described is the third of the young stem, and from it arises the first whorl of roots. When the roots are fully formed, as in the seedling drawn in Fig. 6, a section through their insertion shows the formation called by M. Van Tieghem the 'réseau radicifère[1].' The xylem as well as the

[1] Van Tieghem, Traité de Botanique, 2nd ed., Vol. I, p. 787.

phloem forms a complete circle. This xylem-girdle is thickened at certain points, and on these the central cylinders of the roots are inserted.

Returning to our younger stem (Fig. 4), we pass downwards from the third node into the second internode. In this the bundle *l* has disappeared, for one of its branches has fused with β, the other with γ. Throughout the second internode we have, as shown in Fig. 12, three bundles in the central cylinder which are the direct continuation of the traces from the second leaf, α, β, γ. Surrounding these we have the traces of the first leaf, *A*, *B*, *C*, and in a peripheral circle the cotyledonary traces 1–5. In the succeeding node—the second of the young stem—the traces *A*, *B*, *C* bend inwards to the central cylinder. The lateral bundles *B* and *C* fuse with the lateral bundles β and γ. α forks to right and left, and we again have an incomplete vascular girdle formed. This time, however, it is the xylem of *A* which is distinct from the rest.

The two branches of α ultimately fuse with *B* and *C*. Thus on entering the first internode there are only three bundles within the ring of cotyledonary traces. Here, however, the series of sections which we have been following breaks off. A diagram (Fig. 13) can easily be constructed from other examples to show what will take place in the coming node. But it will be more convenient to consider its structure in detail when we deal with the transition from stem to root. This transition begins almost before the formation of the first node, and continues throughout the short hypocotyl.

Transition from Stem to Root.

The transition from stem to root in the hypocotyl of *Arum maculatum* can be most satisfactorily followed in very young seedlings. Even at the age shown in Fig. 1, all the bundles of the first internode are clearly differentiated. Fig. 14 is drawn from a section passing through the stem-bud of the youngest seedling in our possession. Only two leaves and a growing point are found within the cotyledon, and the

second leaf is quite rudimentary. Yet not only are the bundles of the cotyledon well developed, but three procambial strands are already indicated in the first leaf. A little lower down in the axis these strands are better defined, and the structure of the first internode is perfectly clear. The five cotyledonary traces[1] form a peripheral circle; within them are the three plumular bundles which correspond in position to the traces of the first leaf. But this, as we have seen, is the typical structure of the first internode in much older tubers.

Complete series of sections can be cut through the axis in seedlings of this age without difficulty, and we possess five such sets of transverse sections. All of them begin near the base of the stem-bud and are continued through the tuber into the primary root. Three series are cut through seedlings of the age shown in Fig. 1; two through rather older specimens, corresponding to Fig. 2. The main features of the transition are the same in all these cases, though no two are alike in every detail. Figs. 14 and 15 are drawn from two sections cut from the same axis.

The diagram (Fig. 13) shows that the eight bundles of the first internode form three groups in the succeeding node. But the process is not nearly so symmetrical here as in the second and third nodes. We have five traces entering the central cylinder in place of three, and the transition to root structure begins in the node itself. No part of the hypocotyl therefore shows the characters of a true stem. It will perhaps be easier to appreciate the difficulty of interpretation if we follow the bundles from the first internode through the succeeding node, and the hypocotyl into the primary root in a single instance. We will choose that seedling from which Figs. 14 and 15 are drawn.

It has already been stated that there are eight bundles in the first internode. The five cotyledonary traces lie near the periphery, while the three plumular bundles form a smaller

[1] In Fig. 14 the cotyledon shows six bundles. The sixth is more slender than the others, and disappears lower down. This is a common anomaly.

circle within them. The lateral bundles of the plumule are merely procambial strands, but the third is the direct continuation of the midrib from the first leaf, and its xylem is partially lignified. This bundle we will call *A* to distinguish it from the others, numbering the cotyledonary traces as in Fig. 14.

As we move downwards through the first internode, the cotyledonary traces turn inwards, and at the same time 1 approaches 2 and 5 approaches 4. Near the base of the internode the plumular bundles spread slightly outwards until a section is reached in which all the bundles of the internode lie on the circumference of a single circle. They do not long remain separate from each other. The bundle *A* forks, one bundle fusing with its right-hand neighbour (5), and the other with that on its left hand (1). The two lateral strands of the plumule fuse with 2 and 4 respectively. We have thus five bundles left, corresponding in position with the five cotyledonary traces.

Up to this time all the bundles in the axis have been of the stem-type, the phloem-groups lying outside the xylem and on the same radius. The xylem itself is centrifugal; that is, the protoxylem is internal. But already the transition to a root-structure has begun. The bundles, which now lie close to each other near the centre of the section, have gradually assumed the characteristic appearance shown in Fig. 15. The four lateral bundles form a crescent in front of the midrib. The xylem of the midrib is here, as elsewhere, perfectly distinct from the other xylem-groups. These features, as we have seen, are characteristic of every young node. But the phloem of the midrib (*ph*) is clearly branching to right and left, and will ultimately join two phloem-groups already partly formed near the horns of the xylem-crescent. A third phloem-group is indicated at the place marked * on Fig. 15, and it will divide the xylem-crescent into two parts. All the phloem of the central cylinder will finally divide itself among these three groups, leaving none external to the xylem. In this way the alternate arrangement of xylem and

phloem characteristic of root-structure is attained. It is clear that the root will be triarch.

It would be hopeless to try to follow the 'rotation' of the xylem, the process by which the protoxylem becomes external, in the shapeless groups forming the crescent. But the xylem of the midrib shows it clearly enough. In the particular case we are considering, this group of xylem has assumed the form of a crescent, with the protoxylem-elements in the centre. Each horn is formed of two or three larger vessels (Fig. 15). This little crescent is at first placed symmetrically with reference to the larger one. Gradually, however, it rotates in such a way that one horn at last points outwards, the other directly inwards. It will be seen that in the figure this rotation has already begun; the two larger elements which form the left-hand horn of the xylem-crescent are nearer the periphery of the section than those which form the right-hand horn. Ultimately the two left-hand vessels become completely external, and then die out. In this way the protoxylem-elements (*px*, Fig. 15) are left external to the remaining elements of the xylem. While this has been going on, all the bundles of the stele have drawn much closer together, and one or two larger vessels have been differentiated from the conjunctive tissue within them. In this way a xylem-plate is formed.

It is clear from this description that the transition from stem to root structure has taken place according to the third type described by M. Van Tieghem[1]. The xylem-groups have remained *in situ*, 'rotating' through 180°, in order that the protoxylem may become external; and the phloem-groups have branched to right and left, the adjacent branches of two groups uniting. The branching of the phloem takes place with great regularity in all the specimens we have examined. But it is merely formal to describe the process by which the protoxylem has become external as a rotation. Sometimes indeed, as in the example just described, a partial

[1] Van Tieghem, Traité de Botanique, 2nd ed., Vol. i, p. 783.

rotation does take place. It is supplemented by the disappearance of external xylem-elements, and by the formation of new ones within the protoxylem. In other cases no rotation whatever occurs. The xylem-groups are all approaching a common centre during the transition, and the larger elements commonly move inwards more quickly than the protoxylem, thus leaving it external. In every case we have examined, some of the xylem elements die out of each bundle, and a few larger vessels are formed within the stele. Thus in one way or another the protoxylem becomes external during the transition, but never by a simple process of rotation, and often without any true rotation at all.

The four other seedlings from which we have cut complete series of sections show much variation in the structure of the mode. In all, the xylem-group of the midrib remains distinct, and is continued into one of the xylem-groups of the triarch root. The characteristic xylem-crescent is also more or less completely formed opposite the midrib in every case. But in two cases this crescent shows two xylem-groups, in one (besides the example described) it shows four, and in one seven. This last case is remarkable for a still more considerable deviation from the type. The root-stele when first formed is tetrarch, the fourth xylem-bundle corresponding in position to the stem-bundle we have called *A*. A little lower down, however, this xylem-bundle disappears from the root-stele, and the phloem-groups on either hand of it unite. Thus the root in the end becomes triarch. This variation is probably not uncommon, for in three series of sections through the lower part of the tuber, and the upper part of the root which we have cut through older seedlings, two show a tetrarch structure when the root is first formed. In one of these cases the tetrarch root becomes triarch later on; in the other, the tetrarch structure seems to persist.

We are inclined to consider those cases normal in which three distinct groups of xylem are formed in the first node and are continued downwards into the triarch root. These three

groups would represent three primitive bundles belonging to the cotyledon, two of which branch immediately on entering the first internode. Thus the symmetry of the root-structure would be immediately derived from that of the cotyledon. The anomalies just described show, however, that one of the plumular bundles may occasionally exercise some influence on the root-structure. We hope to obtain some light on this point from the examination of seedlings belonging to allied genera.

EXPLANATION OF FIGURES IN PLATE XXV.

Illustrating Mrs. Scott's and Miss Sargant's paper on *Arum maculatum*.

Abbreviations: *s.*, seed; *t.*, tuber; *s.l.*, scale-leaf; *r.*, root; *c. r.*, contractile root; *b.*, bud.

Fig. 1. *Arum maculatum*. Seedling. Jan. 29, 1896.
Fig. 2. ,, ,, ,, Showing the tuber, radicle, and root-hairs.
Fig. 3. ,, ,, ,, A little more advanced. April, 1896.
Fig. 4. ,, ,, ,, Showing tuber much enlarged. May 7, 1896.
Fig. 5. ,, ,, June 9, 1896. The seed-coats and cotyledon have been cut off by periderm.
Fig. 6. ,, ,, Oct. 14, 1896. The radicle has shrivelled and new roots have been formed.
Fig. 7. ,, ,, Young plant third season.
Fig. 8. ,, ,, June 9, 1898. Young plant fourth season, showing two contractile roots.
Fig. 9. ,, ,, Section through petiole of the innermost leaf of mature plant (Fig. 10), showing the inflorescence enclosed in it and the next year's vegetative bud (*b*).
Fig. 10 ,, ,, Mature plant with vegetatively produced young plant (*A*) attached, and numerous tubers about to form new plants.

Figs. 11, 12, 13. Diagrams illustrating the course of the bundles in the epicotyle donary stem of a seedling about the age shown in Fig. 4.

1–4, Cotyledonary traces.
A–C, Traces from first leaf.
α, β, γ, Traces from second leaf.
l, Mid-rib of third leaf.

Fig. 14. Transverse section of stem-bud, passing through the growing point. From seedling of age shown in Fig. 1. Six bundles in the cotyledon; one of them—which will disappear later—is very slender. Three bundles in the first leaf.

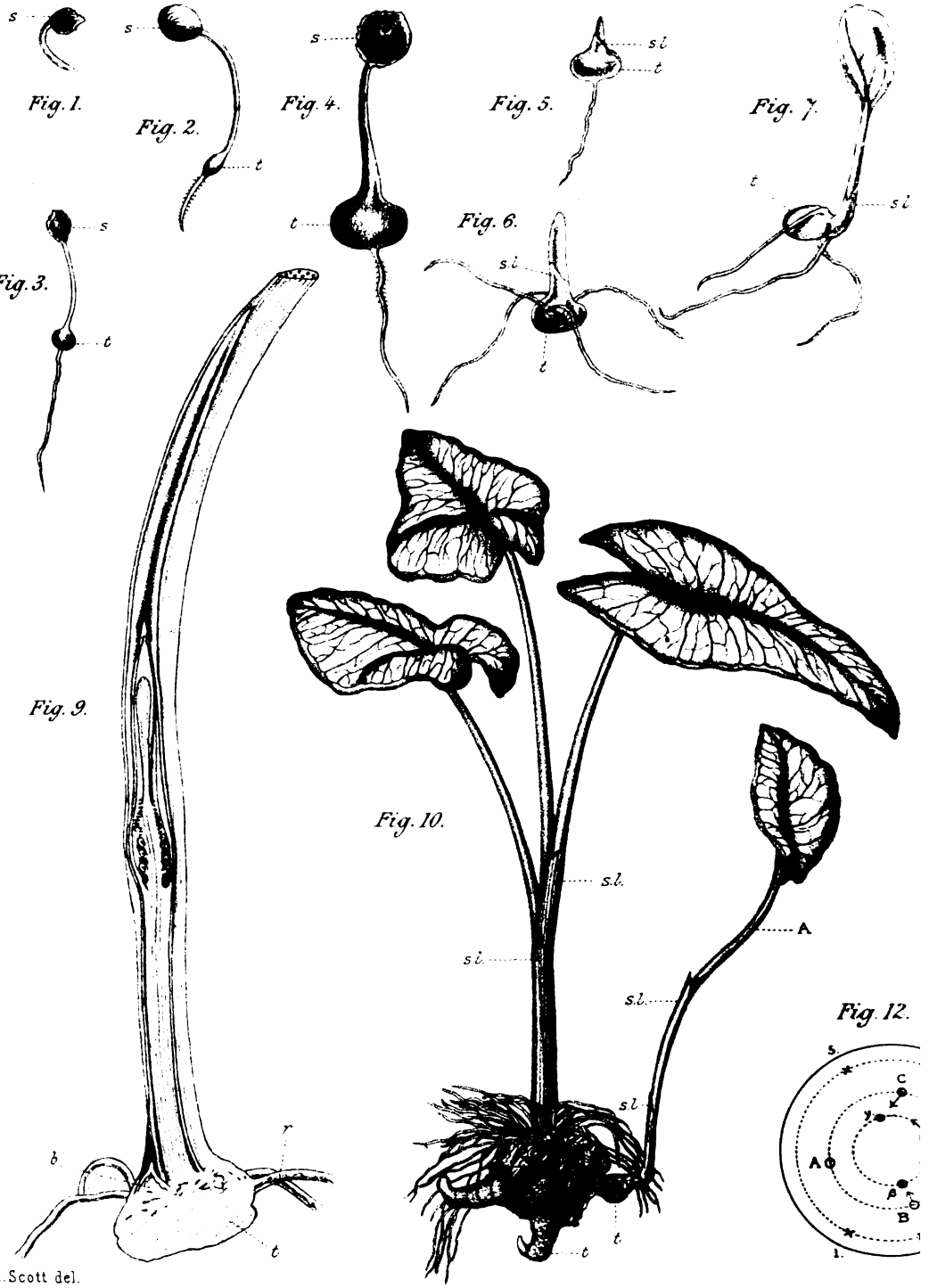
Fig. 15. Transverse section through central part of hypocotyl, showing transition from stem to root structure. Drawn from same seedling as Fig. 14.

ph., phloem of midrib which is branching to right and left.

px., protoxylem of midrib.

x, *x*, *x*, *x*, four groups of xylem forming crescent in front of midrib.

Figs. 1–9 are drawn by Mrs. Scott; Fig. 10 by Mr. G. T. Gwilliam; Figs. 11–15 by Miss E. Sargant.



R. Scott del.

SARGANT AND SCOTT. — GERMINATION OF ARUM.

Fig 8

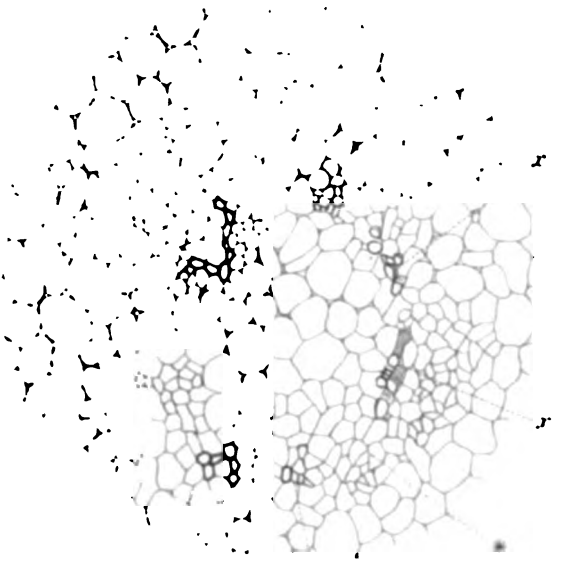


Fig 15.

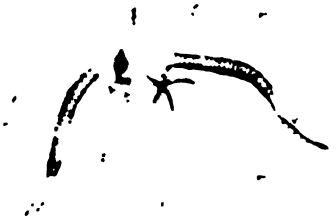


Fig 11

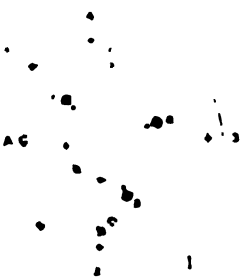


Fig 13

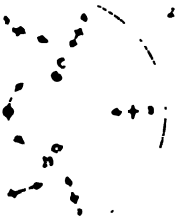
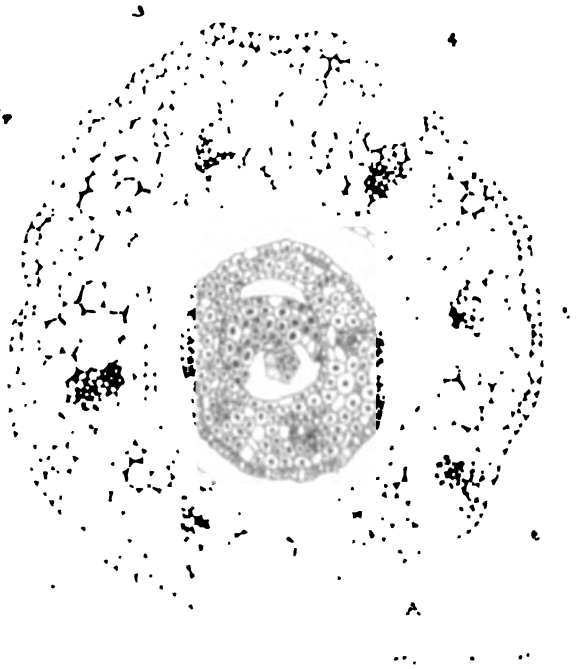


Fig 14



NOTES.

THE ACTION OF CHLOROFORM ON CO_2-ASSIMILATION. —In 1878, Claude Bernard [1] observed that chloroformed water-plants ceased to evolve bubbles of oxygen in the light but that they recovered this power if the chloroform was at once removed. In Schwarz's [2] experiments, however, the evolution of bubbles ceased only when the plants were fatally affected and had lost the power of recovery, and similar results were obtained by Pringsheim [3]. Bonnier and Mangin [4] found that in certain Phanerogams, by using measured quantities of ether, CO_2-assimilation might be stopped without the respiratory activity being affected, and I have recently [5] shown that the prolonged action of ether-vapour causes the chloroplastids to become temporarily or permanently inactive. Ether may therefore not only directly render CO_2-assimilation impossible, but may also finally induce a condition in the chloroplastid during which it is unable to assimilate even when returned to normal conditions. As regards the effect of chloroform further research was evidently necessary, especially since Kny states that filaments of *Spirogyra crassa* immersed for 5 hours in a mixture of one part of saturated chloroform-water to five parts of tap-water showed clear signs of death, but nevertheless were still capable of CO_2-assimilation [6].

Spirogyra nitida (?) was entirely killed after being kept for 8 hours in a saturated solution of chloroform in water, and no evolution of

[1] Claude Bernard, Leçons sur les phénomènes de la vie, 1878, p. 278.

[2] Schwarz, Bot. Unters. aus Tübingen, 1881, p. 102.

[3] Pringsheim, Sitzungsb. d. Akad. d. Wiss. zu Berlin, 1887: 'Die Abhangigkeit der Assimilation gruner Zellen von ihrer Sauerstoffathmung.'

[4] Bonnier and Mangin, Ann. Sci. Nat., Ser. vii, t. iii, 1886, p. 14.

[5] Ewart, Journ. Linn. Soc. Bot., Vol. xxxi, 1896, p. 408.

[6] Kny, Ber. d. D. Bot. Ges., 1897, Bd. xv, p. 401.

oxygen was observed from any of the cells even though the chlorophyllous contents retained an almost normal green colour. If kept for the same time in a semi-saturated solution of chloroform in water, many cells are still living and plasmolysable; but either no evolution of oxygen or in a few cases a faint or doubtful one is shown on examination. Next day a distinct to moderately active power of CO_2-assimilation is shown by living cells, but these are extremely few in number, most having died. Kny largely employed as test-bacteria those taken from putrescent fluids containing meat, and moreover did not consider that it was necessary to ring the preparations in order to exclude external oxygen. Under these conditions however there is a serious liability to error, for such fluids almost always contain facultative or partial anaerobes which continue to move in the absence of oxygen and are attracted by the nutritious substances exuded from dying cells. Such movement continues in the darkness though it may appear as if it recommenced immediately the preparation is exposed to light.

The same author also states that cells killed by acid and by the action of strong induction-currents might continue to assimilate carbonic acid, and in the latter case with an increased activity! A possible explanation of these results has already been given [1], and it seems almost incredible that so keen an observer as Kny could have been led into so palpable an error. The actual experimental work seems however to have been performed by Kny's [2] assistant, and it is hardly necessary to emphasize the fact that the delicate bacterium-method can only be trusted to yield accurate results when it is properly applied by a capable experimenter.

Elodea is a much more suitable plant for experimentation than *Spirogyra*; and if a plant of *Elodea* is kept for one day in a saturated watery solution of chloroform, all the cells are killed and no trace of an evolution of oxygen from them can be detected. A plant suddenly saturated with a watery solution of chloroform containing a slight excess of the latter in the form of a fine emulsion, becomes covered with gas-bubbles if exposed to sunlight, and from the cut end of the stem bubbles derived from CO_2-assimilation continue to escape actively for five minutes, then slowing and ceasing in the succeeding

[1] Bot. Cent.-bl. 1897, Bd. lxxii, No. 9 (Relations of Chloroplastid and Cytoplasma).

[2] Kny, l. c., p. 403. Bot. Cent.-bl., 1898, Bd. lxxiii, p. 439.

five minutes. The chloroform appears at first to exercise a physical action favouring the formation of gas-bubbles, and the heating effect of the sun's rays may also aid in causing an evolution of bubbles after the plant has been fatally injured, for after five minutes immersion the plants have lost the power of recovery and rapidly die in fresh water. By using more dilute proportions and longer periods of exposure it is possible to cause a cessation of CO_2-assimilation without the power of recovery being lost.

Elodea canadensis. (*a*) Plant exposed to sunlight in water at 18° C., super-saturated chloroform-water allowed to trickle slowly in, and the water gently agitated. Evolution of bubbles slows after ½ hour and ceases in 1 hour. Water rises to 28° C. in this time, and hence intercellular air (⅛ c. c.) is increased by ½ c. mm. in volume, an appreciable fraction of the total amount of gas evolved but probably partly compensated for by the increased respiratory activity. Leaves washed and immediately examined, show no rotation, no evolution of oxygen, and the chloroplastids do not take an apostrophic position in strong light. Next day the plant shows feeble evolution of gas-bubbles: on examination about half the leaf-cells and nearly all the stem-cells are living and show with the bacterium-method a moderately active evolution of oxygen, which in a few cases is feeble or doubtful.

(*b*) Experiment repeated with plant in water at 27° C., evolution of bubbles slows in 10–15 min. and ceases in 20–25. Plants immediately washed and placed in fresh water show no evolution of bubbles after 3 hours, but occasional bubbles after 5 hours; while a leaf when first examined shows no rotation, no evolution of O, and the chlorophyll-grains remain dispersed in strong light, in 3 hours there is faint to moderately active evolution of oxygen and the chloroplastids assume the apostrophic position. Next day moderately active evolution of bubbles is shown, and most of the leaf-cells remain living and show active rotation.

These results therefore confirm the original experiments by Cl. Bernard, and show that a stoppage of CO_2-assimilation may be produced by uniformly distributed anaesthetization if properly graduated to the resistant powers of the plant employed.

A. J. EWART.

BOTANICAL LABORATORY, OXFORD.

THE ACTION OF LIGHT ON MESOCARPUS.—Wittrock in 1878 first observed that the chlorophyll-plate of *Mesocarpus* was able to revolve in the cell. He did not attribute this action to light. In 1880, Stahl published a series of papers in the Bot. Zeitung in which he states—

(1) That in diffused light the chlorophyll-plate places itself at right angles to the incident light.

(2) In strong sunlight the edge of the plate is turned towards the source of the illumination.

(3) On continued insolation the plate—until now straight—becomes a curved figure.

In 1888, S. Le M. Moore in a memoir presented to the Linnean Society states that—

'If a specimen be so arranged that, the plate having been in full face, considerable approaches are making towards the profile position, or *vice versâ*, on plunging now into darkness and examining after a short interval, the movement will be found to have been almost or entirely completed[1].'

Respecting these experiments it has been found that a very short light-stimulus will suffice to turn the chlorophyll-plate either from the vertical to the horizontal position, or vice versa. Which of these effects will be produced depends on the intensity of the light, though to bring about the complete change of position a sufficient duration of stimulation is necessary.

A series of observations were made to determine the effect of stimuli of various duration, ranging from 10 seconds to one just sufficient to cause the plate to revolve through 90°. The experiments were carried out during successive days on which the intensity of the light was fairly constant.

I. *Experiment with diffused light* (*turning the chlorophyll-plate from the vertical to the horizontal position*).

In this case the *Mesocarpus* had been previously kept in the dark before being used. Cells were selected in which the chlorophyll-plates were vertical. A light-stimulus was then given for a definite period of time, and the plant (and microscope) was then darkened. In all cases a control-plant in continuous light was run side by side with the one under experiment.

[1] Journal of Linnean Soc., Bot., Vol. XXIV, p. 370.

In the case of the former (the control) it was possible to estimate the angle turned through during successive intervals of time, such as 5, 10, 15, and 20 [1] minutes after exposure. It was not possible to do this in the case of short-stimulus experiments, as the cells would then have received three additional stimuli, so it was necessary to terminate an experiment on examining the result after the period of darkening. But the course of events could be reconstructed by arranging a series of *Mesocarpus* cells, each of which received the same stimulus, and then examining them one by one at successive

TABLE I.

Time of stimulation in seconds.	Angle turned through at end of				Control. Angle turned through at end of			
	5 min.	10 min.	15 min.	20 min	5 min.	10 min.	15 min.	20 min.
10				25°	10°	50°	—	90°
45	10°	25°	30°	40°	15°	45°	85°	90°
55	10°	25°	35°	45°	20°	50°	80°	90°
60	8°	35°	61°	63°	15°	50°	80°	90°
100	5°	36°	65°	86°	10°	50°	75°	90°
120	10°	32°	65°	90°	10°	55°	80°	90°

Illustrating stimulation of vertical plate with diffused light.

intervals of time. In order to reduce any chance of error which might be due to an accidental deviation from the proper normal, each time-experiment was repeated three times, and the mean of the observations was accepted provided that there was close agreement between the results obtained.

Thus, as it was desired to ascertain what had happened at 5, 10, 15, and 20 minutes after stimulation, twelve microscopes were arranged

[1] It was found, as the result of many observations, that no further effect was produced after the lapse of twenty minutes from the time of stimulation in the experiments with diffused light. This does not hold good, however, with the observations (Series II) on the effect of strong illumination.

in four sets containing three in each, and so every observation was checked three times under the same conditions.

The results of such a series of observations are given in Table I.

The behaviour of cells receiving 10, 45, 55, 60, 100, and 120 seconds stimulus is here given, and on the right-hand side the behaviour of the corresponding controls are tabulated. It was found that the rate of turning through 90° varied somewhat at different periods. During the first five minutes the angle turned through was less than that turned through during the fifth to the tenth and the tenth to the fifteenth minute after stimulation. A decrease then generally occurred till the revolution was complete. This slow movement during the first five minutes would seem to point to a latent period.

It is also interesting to note that those cells which received a stimulus only just sufficient to turn, took *no longer* to do so than those in continuous light. There is then a maximum limit of time-stimulation, and by prolonging its duration no additional visible effect is produced. These points could perhaps be better appreciated by plotting a curve than by tabulation.

II. *Experiments with strong sunlight (turning chlorophyll-plate from the horizontal to the vertical position).*

Cells were taken in which the chlorophyll-plates were horizontal, and by the action of strong sunlight were made to turn into the vertical position. Here the times taken by the controls to turn through 90° were appreciably greater than in the first case, the

TABLE II.

Time of stimulation in seconds.	Angle turned through at end of					Control. Angle turned through at end of				
	5 min.	10 min.	15 min.	20 min.	30 min.	5 min.	10 min.	15 min.	20 min.	30 min.
30	10°	15°	15°	15°	15°	10°	20°	60°	70°	90°
45	10°	15°	20°	20°	20°	10°	15°	45°	80°	90°
90	5°	10°	60°	60°	90°	20°	40°	60°	70°	90°

Illustrating stimulation of horizontal plate with strong sunlight.

difference being about 7–10 minutes, and with very strong light the movement is slower, showing that the optimum strength of stimulus has been surpassed. But the table also shows that a *shorter initial stimulus* than that required in the case of diffuse light was competent to produce the full effect. A change in the rate of turning similar to that observed in the experiments of series 1 occurs also here, and it is shown in Table II; the controls also bear this out.

The effect of different gases was also tried. *Mesocarpus* placed in hydrogen and exposed to continuous diffused light turned in the normal time. Some filaments were also placed in hydrogen and the dark for 50 minutes. On exposure to light, while still in hydrogen, these also turned in response to the stimulus in the normal time.

Carbon dioxide acts as a complete anaesthetic, no movement taking place after an exposure for an hour to good diffused light.

The appearance of the protoplasm in the cell when examined with a high power does not appear to alter during the process of turning. Slight staining with dahlia was tried, but this did not reveal anything further.

The protoplasm is rather granular, and the granules perform a slow streaming movement along the cell-walls, very much after the manner of the circulation in *Spirogyra*.

FRANCIS J. LEWIS.

BOTANICAL LABORATORY,
ROYAL COLLEGE OF SCIENCE, LONDON.

Contributions to a Knowledge of the Morphology and Ecology of the Cactaceae: II[1]. The Comparative Morphology of the Embryos and Seedlings.

BY

WILLIAM F. GANONG, PH.D.,

Professor of Botany, Smith College, Northampton, Mass., U.S.A.

With Plate XXVI.

THE present paper comprises the results of an examination into the comparative morphological development of representatives of the genera of Cactaceae. In this investigation I have had four objects in view:—

1. To discover whether the conclusions reached by a study of the comparative anatomy and development at the growing-point of the adults in this family are sustained by a study of their ontogeny; and in how far the very peculiar morphological features of the adults are present in the embryos, and at what stages they develop.

2. To determine whether the form-conditions of the seedlings answer or not to the form-conditions of the adults;

[1] I. Die Morphologie und Biologie der vegetativen Theile. Flora, 1894, Ergänzungsband, pp. 49–86.

[Annals of Botany, Vol. XII. No. XLVIII. December, 1898.]

and, if so, whether these shapes are the result of adaptation to similar conditions, or are determined by the working back of adaptive characters of the adults, or are due to some other cause.

3. To find whether the young stages show features indicating anything as to the phylogeny of the genera.

4. To find whatever else of interest might come out of a minute study of the young stages of this highly specialized family.

HISTORICAL.

Although there are many references to the germination and the seedlings of Cactaceae in systematic works treating of that family, as well as in other works of a morphological or ecological character, nevertheless no comparative study of the young stages has yet been attempted. It is still nearly as true as it was in 1858, when Labouret wrote: 'Les faits relatifs à la germination sont incomplètement observés.' Doubtless there are two reasons why this family, of such unusual morphological and ecological interest, has been neglected in this respect: first, the great difficulty of obtaining authentic seeds, and the considerable difficulty of raising them; and second, the widely spread opinion, distinctly formulated by Pfeiffer, that the seedlings have no taxonomic value. Pfeiffer in 1837 wrote upon this subject as follows:— 'Diese Beobachtungen lieferten mir den Beweis, dass die Keimung bei allen Cacteen, welche keine wahren Blätter haben, sehr ähnlich ist, und dass die Gestalt ihrer Cotyledonen keinen Gattungscharakter abgeben kann. Denn:

(1) Ist dieselbe bei allen Mammillarien und Melocacten ganz gleich, wie ich bereits an einer grossen Menge von Arten beobachtet habe;

(2) Die Form derselben bei *Echin. ingens* unterscheidet sich auffallend von der der übrigen bekannten Echinocacten;

(3) Unter den jungen Pflänzchen von *Echin. robustus*, von welchen ich eine ziemlich beträchtliche Menge besass, war die grösste Verschiedenheit der Gestalt, weshalb ich auch drei

dieser Formen auf ziemlich gleicher Entwickelungsstufe abzeichnete.'

The evidence given in this paper shows that these deductions of Pfeiffer's are incorrect, but they have doubtless had weight with other systematists; and morphologists, for the other reason given, have hardly yet taken them up.

The first writer to describe and figure seedlings of this family was De Candolle, who in 1827 gave a drawing ('made in 1800') of a seedling of *Cactus Melocactus* (*Melocactus communis*), which unhappily contains a morphological error, as pointed out by Miquel in 1839. From that time on to the present, figures and descriptions have from time to time appeared, though in no great abundance, of which I need mention here only the more important, more especially since I have attempted to enumerate all these figures in the special part of this paper.

The earliest account of the process of germination in Cactaceae that I have found was given in 1836 by Zuccarini, who described it correctly in *Mamillaria* and *Echinocactus*. In the next year Pfeiffer figured and described several forms, and briefly discussed their germination in the passage I have quoted above, to which he added other remarks of less importance. Labouret, in 1858, devoted five and a half pages to the description of the germination of eleven species, but gave no figures. Klebs, in his work on Germination in 1885, gave a few observations upon this family. The various papers of George Engelmann figure many embryos as taken from the seed, but rarely figure the seedlings. Irmisch, in his paper on *Rhipsalis Cassytha*, has given the most satisfactory account of the germination of the seedlings of a species of this family which we possess. Goebel has given the fullest account of the seedlings which has yet appeared, including several of the best figures yet published, and a discussion of their ecologic and phylogenetic significance. Lubbock has described and figured several species. Schumann has said but little upon this phase of a subject on which he is such an authoritative writer. In a short paper by Mr. C. F. Maxwell

a few seedlings are described and sweeping conclusions drawn upon altogether too scanty evidence. There are of course other references, including those in inaugural dissertations by Kaufholz and Michaëlis, and in the 'Monatsschrift für Kakteenkunde,' but they are of minor importance. One characteristic of nearly all of the figures in the various works I have cited is this—they show the epicotyl very well developed, but not the form in its true embryo condition; and hence they are less instructive in the present study than would otherwise be the case.

MATERIALS.

The value of a study like the present depends very largely upon the authenticity of the seeds used; and the difficulties of obtaining and raising them are great. I have tried first of all to obtain seeds collected in the field by botanists who are known to be particularly well acquainted with their own flora, and for seeds collected under these conditions I am greatly indebted to Mr. S. B. Parish of San Bernardino and to Professor J. W. Toumey of the University of Arizona, both of whom have most liberally responded to my troublesome requests. Other materials have been sent me by Mr. C. R. Orcutt of San Diego, Mr. W. Fawcett of Hope Gardens, Jamaica, and by a few others, to all of whom I extend here my sincere thanks. Next to material from this source, I have valued that obtained from Botanic Gardens, particularly from those of Palermo and Florence, where the Cactaceae grow under conditions far more nearly natural than in the northern gardens, where indeed many kinds do not bloom at all, or only rarely and in single specimens. Finally, I have obtained some seeds from dealers, but in general I have only trusted the correctness of the naming of the seeds I have bought when they grew into seedlings recognizable as of that species, or when from two distinct sources I have obtained what are plainly the same seedlings; and indeed I have tried to apply this test also to those obtained from Botanic Gardens. One source of error in raising them must be guarded against—

an occasional mixing which happens through careless watering where several pots stand together; the water carrying seeds may splash from one pot into another. It is not impossible that some of the remarkable cases of seeming polymorphism in seedlings described by Pfeiffer and others may be due to a mixing of distinct kinds; and a wrong identification of a given seedling might thus readily come about. In obtaining the seeds I have tried especially to secure representatives of all the genera, of each of the marked divisions of a large genus, and of the morphologically remarkable species. Of the fresh seeds sent me by botanists there is hardly a case where they failed to germinate, though those which were bought often failed to come up. My experience is in marked contrast to that of Henslow, who comments upon the great difficulty of germinating seeds of desert-plants [1]. After the embryos are well developed, however, it becomes increasingly difficult to grow them, though naturally there are the greatest differences between them in this respect. The transition from embryo to seedling, i.e. the formation of the epicotyl, is a crucial point which some cannot pass. They are very liable to rot at the root, or else to form there much corky tissue, which interferes with their proper nutrition and ultimately distorts and destroys them. Still with reasonable care, it is not difficult to raise most of the species.

A very important question here arises as to how far the seedlings raised in a northern greenhouse correspond to those growing naturally at home, and whether safe conclusions as to the latter may be drawn from the former. At least one author (Schumann) has stated that the seedlings vary too much under these conditions to be trusted. But within certain limits I believe there is but little difference between the domestic and the wild seedlings. First; upon *a priori* grounds we might suppose that in one generation, while there may be changes in those characters, such as length, thickness, colour, which are developed in irritable

[1] Journ. Linn. Soc. xxx. 222.

response to outside conditions of light, moisture, &c., in each generation, yet in those characters which are hereditary, i. e. the ground-form, relative positions of the parts, their order of appearance, &c., there can be no perceptible change in one generation. Secondly, the germination of the seeds at home in the desert must take place at the rainy season, for then only is the necessary water available. Now the conditions of the desert in the cloudy time of the rainy season are not so very different from those of our greenhouses. This similarity is in a manner confirmed by the fact that the young embryos will not grow well if exposed in our greenhouses to too bright light, showing that at home they must grow at first in shade. Thirdly, seeds of the same species have been grown by us under very different conditions of moisture and soil, even to such extremes as sand, peat, and sawdust, and despite this the seedlings vary extremely little from one another, except when 'drawn' through lack of light, or dwarfed through some kind of starvation. Some of these conditions must be as far removed from one another as desert from greenhouse. I think that so far at least as concerns the stages before the seedlings begin to depend upon new food-supply made by their own chlorophyll, they are much alike under all conditions. Fourthly, we have raised many of these seedlings into adult stages; and these, except for too great slenderness in some Opuntias and too little colour in some reddish kinds, are not distinguishable from the wild forms of the same species which we find at home in the desert. If the adult stages thus correspond under long-continued differences of conditions, much more must the young stages under very short periods of difference. I believe therefore that seedlings raised in greenhouses with all the light that is good for them, are safe examples of the seedlings of those species in a wild state, the differences being too small to be appreciable in such a study as the present. Nevertheless I freely admit that such studies as these would be much better carried on in the native homes of the plants, for which a properly equipped

desert-station is essential; and after the tropical gardens have been provided for, this may well next attract the attention of botanists.

GERMINATION.

The present study properly begins with the fully formed embryo in the ripe seeds. The development of the embryo, of the ovule, and of the fruit, is a distinct question on which I expect to make another communication later. The subject has already received some attention from Planchon [1], Payer [2], Guignard [3], d'Hubert [4], but the results are too fragmentary to be of use in this study. In general the seeds in the Cactaceae contain a curved embryo with but a slight quantity of albumen lying against its concave side.

In the germination of the seeds I have seen little that is especially remarkable. On absorption of water the seed-coat is either split open along a part of its length, as in *Opuntia*; or a piece is pushed off valve-like from the micropylar end, as in *Echinocactus*, *Cereus*, &c. In the latter cases, and no doubt in many others, this is effected by the great swelling of a ring of epidermal cells at the lower end of the hypocotyl, which form a distinct ridge or collar from which later the hair-collar develops. This swelling is common in other families, and no doubt forms the basis of the well-known 'wurzel-hals,' or 'haftscheibe.' Klebs has correctly described the nature of the germination in this family; it belongs distinctly to his fifth type, those with the main root growing little or not at all during germination, with a crown of long root-hairs on the swollen base of the hypocotyl, the hypocotyl lifting the cotyledons out of the seed to above ground. In the latter respect this account is not strictly accurate for this family, for the seed-coat is commonly carried up on one of the cotyledons, no doubt to allow of the absorption of the

[1] Ann. Sci. Nat., Bot., 1845, 275–311.
[2] Ann. Sci. Nat., Bot., 1872.
[3] Bull. Soc. Bot. de France, XXXIII.
[4] Ann. Sci. Nat., Bot., 1896.

last trace of the albumen in combination with an early spread of the cotyledons to the light.

As the lower end of the hypocotyl issues from the seed, its positive geotropism manifests itself, and it bends downwards; at the same time from the swollen collar there grows out at about 45° a great number of long slender straight hairs, which attach themselves to the soil or other substratum, forming a series of cords binding the young plant firmly to the soil (see Fig. 17 *c*, *d*). It seems to me that these hairs come out at a constant angle to the stem, and are not geotropic, in which case it is an interesting question how they are guided in assuming that direction. This method of binding the seedling to the soil is well known elsewhere (see Klebs), and is generally considered necessary to hold the seedling in position until its root becomes fixed. But it, like other methods of fastening young germinating embryos to the ground, may have rather the significance of providing a resistance for the root to work against in the mechanical work of forcing its way into the ground, as otherwise the embryo would simply be lifted, and there would be no leverage for the work. The root at first grows very slowly, but the hypocotyl, as it issues from the seed-coat, begins at once to swell at the base, and this swelling extends regularly upward as it issues from the seed. Finally, only the cotyledons, sometimes three in number, are left, and these are still very small; but now either these are disengaged and swell also, or else they remain, one or both, for a time in the seed; sometimes however they seem unable to withdraw themselves from the seed-coat, and they are held and cut by the seed-coat as they swell, though in their more vigorous growth at home in the deserts this probably does not happen. As soon as the hypocotyl is well out of the coat, its upper end manifests its apogeotropism and curves upwards. The embryo, on issuing from the seed, turns first pale green; but very soon, in many species, red colour appears. At first the cotyledons are tightly pressed against one another, but they separate later, and, as the epicotyl begins to grow,

they are forced apart until finally they stand out at right angles to the hypocotyl. Thus is germination completed.

A distinct and important feature of this process is the remarkable and rapid swelling of the hypocotyl, and consequent immediate assumption of a succulent habit. On examination, however, I have found this to be due, not to increase in number of cells through division, but chiefly to the great increase in size of the cells already laid down. In an embryo of *Cereus triangularis*, for example, lying in the seed, every cell is small and full of protoplasm and starch; while in that which is fully germinated the cells are many times larger, each one is rounded, and has a thin film of protoplasm around the wall, whilst their number is very nearly the same in the two cases[1]. It is plain then that germination consists here simply of the absorption of large amounts of water, by which cells already laid down in the ungerminated embryo simply swell to full size, and no doubt with no material increase in dry weight. From this it follows also that up to the time when new substance is made by the chlorophyll in amount sufficient to enable new growth to begin, which period is roughly marked by the beginning of the growth of the epicotyl, there is no line to be drawn between embryo and seedling; in other words, we may best speak of the stage where the embryo is still lying in the seed as that of the ungerminated embryo, and that in which it has come out, turned green, spread its cotyledons, but before it shows the epicotyl, as that of the germinated embryo. It is this marked and important stage of the germinated embryo, with the epicotyl just beginning to show, that I have tried to represent in the drawings which accompany this paper. The stages after the epicotyl is developed may best be called the seedling. The shape of the seed makes the young embryo asymmetrical, but as it swells it becomes more and more symmetrical in form, though it does not, as a rule, become perfectly so.

There is naturally great variation in the rapidity of germina-

[1] The statistics upon this subject are to be given in a paper referred to on a later page.

tion of the different species, and on account of the condition of the seed, time of year, temperature, &c. In most forms, after the completion of germination, there is a pause, and some time elapses before the epicotyl appears: in many kinds which it is easy to germinate, it is very difficult to ensure development into the seedling-stage, which shows that the materials laid up in the seed make them to some extent independent of external conditions, but that when they must begin to rely upon the surroundings for further progress, they do not find these favourable. No doubt in the deserts, germination is more rapid than in our greenhouses, and one may suppose that the seedlings attain a considerable size before their first dry season arrives.

To show the rate of germination, the following facts are selected. On March 20, 1895, series of seeds were planted in a greenhouse under given favourable conditions; they appeared above ground as follows:—*Opuntia basilaris* var. *ramosa*, in five days; *O. serpentina*, nine days; *Echinocactus viridescens*, fifteen days; *O. echinocarpa*, nineteen days; *Mamillaria radiosa* var. *neo-mexicana*, twenty days; *O. tetracantha*, twenty-three days; *Echinocereus phoeniceus*, twenty-seven days; *E. pectinatus rigidissimus*, twenty-nine days; *M. Grahami*, twenty-nine days; *O. phaeacantha*, fifty-two days. Some seeds of the above species did not germinate for a year (*O. echinocarpa* and *E. phoeniceus*), which is a phenomenon well known in other cases, and perhaps has an ecological meaning. None, however, came up in pots kept for a third year. I have made no observations upon the length of time Cactus-seeds retain their vitality. Pfeiffer[1], however, states that seeds of three species sown nine years after ripening came up in fourteen to eighteen days.

The seedling-growth is most rapid in *Opuntia*. In *O. echinocarpa* planted March 20, the epicotyl with leaves was 7 cm. long on June 13, and in May of the next year some were 15 cm. long and 3.3 cm. broad. The growth of *O. serpentina* planted at the same time was nearly as rapid.

[1] Neuere Erfahrungen, p. 122.

The development of the earliest stages of the epicotyl is much the same throughout the family. The cotyledons, however, behave differently in the different genera. In *Pereskia* they apparently fall off; in *Opuntia* and some of the broad-cotyledoned Cerei, and in *Phyllocactus*, they wither up without falling; in the other genera they are persistent, but in the further growth in diameter of the hypocotyl they broaden at the base and gradually become merged into the stem, a curious case of ontogenetic metamorphosis. In the development of the epicotyl, first of all two leaves appear from the growing-point at right angles to the cotyledons; then another pair nearly at right angles to these; then there comes either a fifth in position to begin a $\frac{2}{5}$ spiral, which later may become a $\frac{3}{8}$, as is common in *Opuntia* and *Mamillaria*; or else the second pair is at right angles to the first and the third pair over the first, thus forming four rows, the foundation for four ribs, as is common in the ribbed forms. These ribs may then diminish in number by dropping one or more, or may increase by the splitting of one or more. In the further development of the epicotyl, the peculiar characteristics of the adults appear at diverse stages, a subject which will be treated later in the special part of this paper.

The roots in both embryos and seedlings are very simple, slender, and sharply distinct from the hypocotyl. They rarely become succulent. In some species, as *Echinocactus Wislizeni*, the epicotyl develops very rapidly and the root very slowly, so that the seedling has a curious three-storied appearance—a swollen globular epicotyl above, a cylindrical smaller hypocotyl below that, and the very slender root below all.

Cellular Anatomy.

The cellular anatomy of this entire family is of great interest and is still imperfectly known. I have naturally made some observations upon this subject in the embryos and seedlings, but shall not attempt to treat of it here, for the reason that it is being thoroughly studied by one of my students whose results will before very long appear, and whose paper on the

subject will be in a measure a supplement to the present one. I need only state that the chief characteristics of the cellular anatomy of the germinated embryos through the family are as follows:—epidermis, one layer of cells, thin, often papillate; cortex of very large round cells, sometimes visible to the naked eye, and forming the greater part of the hypocotyl, from four to six times the diameter of the central cylinder (Figs. 2 *d*, 5 *d*); central cylinder very simple, of two to six fibro-vascular bundles. The young root is characterized by a great development of cork, slight development of cortex, and a condensation of the central cylinder. Naturally this simple structure becomes much modified in the seedlings, and still more in the adults.

The Colour-Factors of the Embryos.

The germinated embryos in this family show a considerable range of colours, from a clear typical chlorophyll-green in some Platopuntiae and climbing Cerei, to a deep red in Mamillariae and others. As the embryos lie in the seeds they show no colour in any of the species I have observed, except the usual translucent white. On emerging from the seed, they turn at first pale green, which deepens upon some but in others is soon masked by the red, or, as in Cylindropuntiae, becomes olive or greyish green. In *Echinocactus viridescens*, for example, as it breaks out from the seed, the embryo turns at first pale green, but very soon a blush of clear red appears on the tips of the cotyledons, and spreads over all the upper part of the hypocotyl, and it appears vividly also on the first two leaves of the epicotyl. One of the most brilliantly red species I have noticed is *Mamillaria Nuttallii*. Here and there over these red embryos, one sees brighter greenish spots, which are areas under the stomata. Examination shows that the red colour is in the sap of the epidermal cells. Moreover its appearance is dependent upon light, for not only does it appear only on parts exposed directly to the light, even to the extent of forming only upon the upper side of a hypocotyl when this is lying upon its side, but in

E. ingens, which is normally very red, no colour at all appeared when I placed caps of tinfoil over the embryos as soon as germinated. The appearance of the colour, therefore, is a clear case of irritable response to the action of light as a stimulus. But there is another distribution of colour which is noticeable. In *Echinocactus Wislizeni*, *Opuntia Engelmanni occidentalis*, and some other species, the colour is not general, but is limited to a single bright red spot on the outside of the base of the cotyledons; and moreover the leaves of the epicotyl (particularly in *Cereus grandiflorus*) show the same feature. I think this is the result of the appearance of the colour only at the time when the leaves are very young and still folded with their faces against one another and their backs only exposed to the light. But it is not confined to the leaves, for the colour in some species only runs in streaks in the depressions between the ribs of the stem [1].

In seeking an explanation of the presence of the red colour, we must recall the fact already stated that it appears only in response to light as a stimulus; and it is most intense in the forms which live exposed to the greatest brightness. It does not appear at all in *Pereskia aculeata*, the climbing species of *Cereus*, *Phyllocactus*, *Rhipsalis*, and those Platopuntiae growing in the less extreme deserts, such as *Opuntia vulgaris* and *Rafinesquii*, and it is best developed in the most extreme desert-forms of *Mamillaria*, *Anhalonium*, and *Echinocactus*. In other words, the more mesophytic the habitat, the less the colour; the more xerophytic, the more the colour. There are two explanations of similar red colour elsewhere. First, according to Stahl, it may serve to absorb some of the light-rays, and convert them into heat which is of use in the processes of growth at a time when there is none too much heat available. Secondly, it may serve as a light-screen, cutting off the rays of the violet end of the spectrum, which act injuriously upon the living tissues. On the whole, in

[1] The spot of colour at the base of the leaf is not uncommon elsewhere. I have noticed it on the base of the petiole in *Lysimachia alnifolia*, in a species of Maple, and elsewhere; also in *Salicornia herbacea*.

view of its distribution, the latter seems far more probable than the former as an explanation of the colour in these embryos. It is true the colour is present in the wet season, when the weather is clouded, but it is not unlikely that even then there are periods of vivid sunshine. But in the absence of exact observations of the subject in their native homes, these theories, like many others relative to the ecology of desert-plants, are mere guesses.

The Size-Factors of the Embryos.

Comparing the germinated embryos throughout the family, we find a wide range of size, from *Pereskia* on the one hand to *Mamillaria* on the other (see figures; *Pereskia* and *Opuntia* are drawn natural size, all others magnified two and a half times). In seeking for the influences which determine the size in the different cases, we naturally turn first to ask whether size in the germinated embryo is related in any way to size of seed from which it comes. Excluding now certain cases presently to be considered, in some species of *Cereus*, *Phyllocactus*, and *Opuntia*, where there is a special growth of the cotyledons, it is plain that there is a relation between these two. It not only shows in a comparison of seed and embryo, but it follows also from the fact I have mentioned that the growth of the embryo to its fully germinated stage is little more than a swelling to full size of cells already laid down in the seed. The question then resolves itself into this—is it, in the main, the size of the seed which determines that of the germinated embryo, or is that of the embryo the more important factor, the seed being the size it is in order to accommodate it? If one compares the size of embryo or seed with the size of the adult plant throughout the family, it appears at first sight as though there were no constant connexion between these two. *Cereus giganteus*, for example, has a seed no larger than many of the smaller Cerei. But if, instead of particular species, we consider groups such as genera, we find that there is a relation between the average

or prevailing size of adults in the genus and that of the seeds and embryos. Thus *Pereskia*, including great shrubby climbers, has the largest embryos; next, Opuntias have the largest adults, seeds, and embryos; *Cereus* and *Echinocactus* are in all three respects much smaller, the latter more so than the former; *Mamillaria* and *Anhalonium* are in all respects smallest of all. Considering the natural relationships of these groups, we may say that the seeds in the Cactaceae have phylogenetically grown progressively smaller, just as the adult plants have done, taking the embryos with them. In general, though with some exceptions, this progressive diminution in size of the adults accompanies an increasing dryness of habitat. It is then probably true that the size of the embryos is reduced, not directly by the dryness, but that it accompanies the reduced size of the adults and seeds; for if the former were true we should find much greater variation in size of the embryos than we do. A marked and important exception to the reduction in size is found in the climbing Cerei and *Phyllocactus*, where the embryos are much larger than those of the desert Cerei, and have leaf-like cotyledons. But the forms possessing these larger embryos have abandoned the desert for a life in the woods, where the mesophytic conditions allow of a much larger spread of surface, and in this case it is plain that the embryos themselves respond to these conditions and increase in size, a point which will be discussed later.

Another and important incidental feature of size in the embryo is its increase when the epicotyl is not allowed to develop. In my plants, sometimes by accident and sometimes by design, the epicotyl and axillary buds of the cotyledons became removed; in such a case no new buds formed, but the embryo continued to increase in size until it became double that of the normal (Fig. 2 *e*). What this must mean is, that a certain amount of food-substance which would normally have gone into the building of the epicotyl, is here diverted into making larger the cells of the embryo (cotyledons and hypocotyl). This growth of the embryo under such conditions would be difficult to explain on the

supposition of a specific 'bildungs-stoff' for the epicotyl, but speaks rather for a large power of individual adjustment in the protoplasm of the embryo. The phenomenon is, of course, well known in other cases [1], and is, I suppose, comparable with the effects of castration in animals.

The Form-Factors of the Embryos.

If we view now the forms or shapes of the embryos at the stage when germination is completed, and the epicotyl is about to appear, we find an immense range of form from the very un-Cactus-like *Pereskia* (Fig. 1) on the one hand to the nearly globular, almost cotyledonless *Mamillaria* (Fig. 47) on the other. As this subject constitutes the most important part of our present inquiry, I must here treat it in some detail, and shall take up the genera in succession. At the same time I shall describe the development of the epicotyl when it is noteworthy, and add remarks upon other features of importance. In addition to references to figures accompanying this paper, I shall mention all other figures of germinated embryos known to me, excepting a very few which are worthless, so that in this respect I mean this work to be monographic. Finally, I shall add a summary of what may be deduced as to form-factors.

1. Genus **Pereskia**.

P. aculeata, Mill. Figure 1.
P. Pititache, Karw. Figure 9, Pl. II, in Zuccarini.
P. Bleo, DC. Figure (embryo in seed), Pl. LXIII in Schumann, Flora brasiliensis.

This genus consists of climbing woody forms with perfectly developed leaves, merging over to upright succulent species, approaching and perhaps merging with Cylindropuntiae. The embryo (Fig. 1) of *P. aculeata* has thin, very leafy cotyledons, showing distinct netted veins; their asymmetry is due to their position in the seeds, which are very flat. As they grow they become jointed at the base, precisely as are

[1] Jost, Prings. Jahrb. 1895; also very easy to produce in young Bean-plants.

the later leaves, and probably like them they are shed at the dry season. The plumule shows first a leaf like the later ones, then another, and so on alternately. As the cotyledons lie in the seed they are flat, and hence very different from those of *P. Bleo* as figured by Schumann. There is no doubt of the identity of my seeds, for one of them grew into a seedling of this species. *P. Pititache*, as figured by Zuccarini, has an embryo resembling more nearly *Opuntia*, though the adult is described as woody. Probably the embryos of some of the succulent species, as *P. spathulata*, will be much like those of *Opuntia*. This mesophytic type of germinated embryo in *P. aculeata* is of course correlated with its growth under mesophytic conditions. No spines are produced in the young seedling, though the axillary buds produce the multicellular hair-like structures which are probably homologous with them.

2. Genus **Opuntia.**

O. bernardina, Engelm. Figure 2.

O. echinocarpa, Engelm. and Bigel. Figure 3. Also in Engelmann, Pl. XXIV.

O. Whipplei, Engelm. and Bigel. Figures 9, 10, Pl. XXIV, in Engelmann.

O. vulgaris, Mill. Figure 5. Also Fig. 57 G in Schumann (Engler and Prantl). Also Ganong in Botanical Gazette, XXV, Pl. XVI.

O. Rafinesquii, Engelm. Figure 7, Pl. XXIII, in Engelmann.

O. missouriensis, DC. Figure 17, Pl. XXIII, in Engelmann.

O. tortispina, Engelm. and Bigel. Figure 5, Pl. XXIII, in Engelmann.

O. basilaris, Engelm. and Bigel. Figure 398 in Lubbock, II, 11.

O. basilaris ramosa. Figure 4.

O. Engelmanni occidentalis, Salm-Dyck. Figure 7. Figure 400 in Lubbock, II, 12.

O. Ficus-indica, Mill. Figure 6. Also in Schacht, Lehrbuch der Anat. u. Phys. der Pflanzen, Fig. 472, Pl. II (not seen).

O. sp. ? Figure in Zuccarini, 8, Pl. II.

Very numerous beautiful figures of ungerminated embryos of species of *Opuntia* are given in Engelmann's works.

This genus, nearly related to *Pereskia*, consists of shrubby, branching, jointed forms, falling into two groups:—the Cylindropuntiae, living in dry deserts, with cylindrical joints and usually subulate leaves which may be up to an inch in length; and the Platopuntiae, with flattened joints becoming true phyllocladia, smaller leaves, and as a rule occupying less

extreme desert situations than the Cylindropuntiae, some of them growing even in woods. Many lines of evidence show that the Cylindropuntiae are the stem-group from which the Platopuntiae are an offshoot.

Turning to the embryos, we find that all have a slender, non-succulent hypocotyl, and well-developed, though fleshy, cotyledons approximately the length of the hypocotyl. With respect to the exact form of the cotyledons, however, two types are distinguishable;—some, such as *O. bernardina*, *echinocarpa*, *serpentina*, and *tetracantha*, have equal tapering cotyledons, triangular-cylindrical in section (Fig. 2 *c*), becoming flatter with age, shorter than the hypocotyl; while others, *O. vulgaris*, *missouriensis*, *Ficus-indica*, *tortispina*, *Rafinesquii*, *Engelmanni occidentalis*, *phaeacantha*, and other Platopuntiae, have them flatter and more leaf-like (Fig. 5 *c*), unequal, and usually longer than the hypocotyl[1]. This distinction of the thick leaves for the Cylindropuntiae, and the thinner for the Platopuntiae, holds in all species that I know of, with the following exceptions:—as figured in Engelmann *O. Whipplei* seems to have flat cotyledons, but as the plumule is shown developed, and since even in Cylindropuntiae they flatten with age, this may not be a real exception; *Opuntia basilaris* is, however, a real and marked exception, for in Lubbock's figures and my own, the cotyledons are distinctly of the Cylindropuntiae type, though this species belongs to the Platopuntiae[2]. As however *O. basilaris* is an extreme desert-species, it suggests that the form of the cotyledons is determined chiefly by the direct environment, the more condensed form occurring in the more extreme deserts, and the flatter and more leaf-like in moister climates, where the natural tendency of the plant to spread as much leaf-surface as possible is allowed more freedom to produce larger and flatter leaves. This can be tested by observing on the one

[1] The extreme of this flattening is probably reached in *O. brasiliensis*, as shown by Schumann's figure on Pl. 61, Flora brasiliensis.

[2] *O. basilaris* is in several respects a remarkable form; particularly noteworthy is its profuse branching from near the base. My seedlings of 2 cm. length (in the var. *ramosa*) showed no trace of it, though a larger one showed one branch.

hand Cylindropuntiae which grow in the moister climates, and on the other, Platopuntiae which grow in the extreme deserts, but at present I have not material for this comparison. In any case there can be no doubt that the relatively larger and flatter cotyledons of the Platopuntiae do not indicate that they are more nearly related to *Pereskia* than are the Cylindropuntiae, for all evidence is against such a possibility, but they are the result of a re-enlargement allowed by the moister climate in which they grow. Another explanation for the cotyledons of *O. basilaris* is that this form has come off from the Cylindropuntiae entirely independently of the other Platopuntiae, and has retained the primitive leaves.

The differences between the two divisions of *Opuntia* are shown clearly also in the cellular anatomy, but I shall here cite but one phase of this. The Cylindropuntiae have a six-bundled central cylinder, as shown in Fig. 2 *d*, while the Platopuntiae have a two- or four-bundled cylinder arranged as shown in Fig. 5 *d*.

The cotyledons, normally two, are sometimes one, often three; and sometimes, as in *O. vulgaris*, elsewhere described, which is polyembryonic, there may be more, with some imperfect. In Platopuntiae they are unequal in size, and it is easy to see that the longer is on the convex side in the seed, and the shorter on the concave. No doubt this inequality of the cotyledons is not in the least to be traced to adaptation, but is a simple result of position in the seed, which gives less room to one than to the other, hence allowing it to make fewer cells; and as germination is little more than the swelling of cells already laid down, the cotyledons must be unequal. Many Platopuntiae have the cotyledons placed incumbently in the seed, while some Cylindropuntiae have them accumbently, and one at first attributes the flat cotyledons of the former and the nearly half-cylindrical form of the latter to this position: but an inspection of the many fine figures of embryos and seeds given by Engelmann disproves such a connexion.

The cotyledons have axillary buds, which I have made to

develop by removing the epicotyl in *O. bernardina* and one or two other species. A remarkable feature occurred in one seedling of *O. echinocarpa* (Fig. 3 *c*). One of the cotyledons, possibly in reality two congenitally united, was forked near its tip, and in the fork bore a bud producing hairs and spines of an altogether normal sort, which persisted long after the epicotyl developed. Such a case is difficult to explain on the basis of a formal morphology, for leaves, which the cotyledons undoubtedly are, do not produce buds normally in this family.

The development of the epicotyl is simple, and is as described earlier for the family in general. From the first, the general mode of formation of leaves and axillary buds agrees with what is found in the adults, except that the characteristic bristles (Borsten), the remarkable hair-sheath of the spines in Cylindropuntiae, and the nectar-glands formed of metamorphosed spines are all absent from the earliest clusters and appear later, though I have not determined exactly when nor how.

The epicotyl in all species of this genus is at first cylindrical. In *O. vulgaris* and other Platopuntiae it is only when the epicotyl is at least a centimetre long that it begins to flatten, and then it is parallel to the faces of the cotyledons. It would be of much interest to determine whether in the seedlings, as in the young shoots described by Goebel[1], the flattening is dependent upon the presence of light, and hence is a phenomenon of irritable response in which the growth-effect has not yet become hereditary.

The epicotyl in its development forces apart the cotyledons, which however retain a connexion with one another at their bases, so that the epicotyl seems to rise from a sort of sheath. When the epicotyl and the axillary buds of the cotyledons are removed, the hypocotyl grows far above the normal size, but the cotyledons wither as they do under normal conditions (Fig. 2 *e*).

[1] Flora, lxxx, 98.

3. Genus Cereus.

C. Thurberi, Engelm. Figure 8.
C. giganteus, Engelm. Figure 9.
C. peruvianus, Mill. Figure 10.
C. Hystrix, Sweet. Figure 11.
C. Bonplandi, Parm. Figure 12.
C. Martianus, Zucc. Figure 13.
C. grandiflorus, Mill. Figure 14; also Fig. 5, Pl. II, Goebel, Schilderungen, I.
C. nycticaulis, Link. Figure 15.
C. spinulosus, DC. Figure 16.
C. triangularis, Mill. Figure 17.
C. subrepandus, Haw. Figure 10, Pl. XVI, in Pfeiffer.
C. eriophorus, Hort. Figures 4, 5, 6, 7, Pl. II, in Zuccarini.
C. Gregii, Engelm. Figure, Pl. LXIII, in Engelmann.
C. Emoryi, Engelm. Figure, in Lubbock, II, 9.

This genus consists of stout columnar to short almost globular, or else deflexed or creeping or slender climbing forms, rarely jointed, always (excluding *Phyllocactus*) radial, generally ribbed. We recognize, with Schumann in his latest monograph, four main divisions:—*A*, those with erect columnar stems; *B*, those with stems at first upright, but at length declined on the ground; *C*, those at first upright, later leaning upon supports, and thus climbing or hanging but lacking aerial roots; *D*, those climbing and possessing aerial roots. This apparently very artificial division is much more natural than it seems, but is far from satisfactory. It is, however, the best we have. In this family, as Schumann has pointed out, the vegetative characters are more important in classification than those taken from the flowers, which in the large genera are singularly uniform.

Taking *Cereus* as a whole, we find throughout a succulent hypocotyl, with distinct cotyledons, usually broad at the base and set a little apart, with bases parallel. In the relatively large size of the cotyledons they approach *Opuntia*. But in the different divisions there are differences which are fairly constant and which usually distinguish them. Of the important columnar group, good types are *C. Thurberi* and *giganteus* (Figs. 8 and 9). In these two very similar and closely related

forms the cotyledons are as broad as the hypocotyl and about as long, pointed, triangular in section, with the inner line of their bases parallel. In all of these characters they show resemblance and doubtless relationship with the Opuntiae, particularly the Cylindropuntiae, and stand nearer to that group than do any other Cactaceae I have studied. As compared with Cylindropuntiae, these germinated embryos are of smaller size and much more succulent, as is to be expected in a group which upon the whole is more xerophilous in habit. The resemblance is further increased by the common occurrence of axillary buds to the cotyledons in *Cereus*. The epicotyl at first bears the leaves and axillary clusters only upon the $\frac{2}{5}$ system, and the development of ribs comes later, one of the cases numerous in this family in which phylogeny is repeated in ontogeny. The transition to ribs is easy, for these at first are five in number, representing of course the vertical orthostichies of the $\frac{2}{5}$ phyllotaxy.

Another division of columnar Cerei is that including *C. peruvianus* and *C. Hystrix* (Figs. 10 and 11). In both of these the cotyledons are much shorter and somewhat narrower than the hypocotyl, and the hypocotyls themselves are comparatively slender. I cannot explain their great deviation from the *giganteus* type except by supposing that the relationship of these two groups is not so close as has been thought. In *C. peruvianus* the axillary clusters fall into lines, and the ribs, usually five, form at once. In the variety *monstrosus*, which reproduces through seeds, the fasciation did not appear in seedlings under 2 cm. in height.

In division *B* I have had no material, though the *C. Emoryi*, figured by Lubbock, belongs here.

To division *C* belongs *C. Bonplandi* (Fig. 12), in which the form of the embryo, though not of the adult, suggests relationship with *C. peruvianus* and *Hystrix*. This species shows a peculiar feature of the cotyledons, in that one of them is always cleft at the tip, and this is rarely the case with both. This feature occurs rarely in other species, but this is the

only one in which I have found it regular. One supposes at first that it is due to the splitting of the tip of the convex cotyledon against the tip of the concave one in the seed, but careful examination has not certainly confirmed this. Another peculiarity in this species, also unique in my observation, is the intercalary basal growth of the cotyledons after development of the epicotyl, thus carrying the axillary buds away from the latter (Fig. 12 *c*). This suggests the similar basal growth of the tubercles in *Mamillaria*, *Leuchtenbergia*, &c., so important in producing the configuration of those genera. I have made one of these buds develop by removing the epicotyl. On the epicotyl at first four ribs develop, which often become eight, later dropping back to five.

In division *D* occur several forms of much importance. First is *C. Martianus*, which differs so much from others in this division as to raise the suspicion either that my material is wrongly named, or else that the species does not belong to this division. The germinated embryos (Fig. 13) appear to stand about intermediate between the columnar *giganteus*-like forms and the climbing species now to be described.

In *C. grandiflorus* and *nycticaulis* we have two climbing species very closely related, and very much alike both in adults and embryos (Figs. 14 and 15). In both of these, and also in *C. spinulosus* and *triangularis* (Figs. 16 and 17), two other climbing and not-distantly related forms, the cotyledons are unequal, flat and broader than the hypocotyl, though they are not broadest at base as are most other Cerei, but a little above it. One notices in these cotyledons a resemblance to those of the Platopuntiae, but only a little reflection is needed to show that this resemblance is not genetic, but ecological. These climbing Cerei live under conditions more mesophytic than xerophytic; and as all evidence shows that they have been derived from forms more like the columnar species, this larger size of the cotyledons cannot be primitive, but must have been re-acquired in response to conditions which permit of that increase of surface towards which green parts are continually tending. They are of

a clear green in all of these species, and large enough to do no inconsiderable amount of leaf-work, and wither with age instead of merging with the stem. They have good buds in their axils. Another feature which the embryos of these species have in common, and which may be correlated with their habitat, is the rapid growth of the hypocotyl and the relatively slower development of the cotyledons. In *C. nycticaulis* and *triangularis* the epicotyl forms first two leaves at right angles to the cotyledons, then two at right angles to these, thus forming four rows giving origin to four ribs. In *C. nycticaulis* one of these ribs soon splits into two, and the five then distribute themselves over the entire circle. This formation of new ribs by the forking or splitting of old ones is common in this family, indeed is the common method of forming the new ribs, and it shows how the rib once formed becomes, as it were, a true morphological element. In *C. triangularis*, on the other hand, one of the ribs soon stops abruptly; the one opposite to it keeps on its course, while the two intermediates turn spirally until they evenly occupy the circumference, after which the three persist through the life of the plant. In this early and temporary presence of four or five ribs in a characteristically three-ribbed species, we have another good case of repetition of phylogeny in ontogeny, a principle not very frequently illustrated by plants.

In *Cereus* the morphological composition of the adults is extremely simple. The same axillary points produce first a cluster of spines and later a branch or flower. Hence nothing of special importance is to be expected in the seedlings, for this is practically the kind of axillary bud which the seedlings produce throughout the family.

4. Genus **Phyllocactus.**

P. Ackermanni, Walp. Figure 18.
P. anguliger, Lem. Figure 19.
P. phyllanthoides, Link, *grandiflorus*. Figure 20.
P. Phyllanthus, Link. Figure 21.
P. stenopetalus, Salm-Dyck. Figure in Goebel, 104; also Lubbock, II, 9.
P. latifrons, Walp. Figure 6, Pl. II, in Goebel.
P. Phyllanthus x *C. flagelliformis*. Figure in Pfeiffer, XI, Pl. XVI.

This genus, which merges with *Cereus* through the climbing triangular species (like *C. triangularis*), contains mostly epiphytic forms in which the ribs are reduced to two, resulting in flat phyllocladia (with axillary buds only upon the margins), which finally become thin and leaf-like upon cylindrical stems. These phyllocladia are set vertically, which probably comes about through suppression of the lateral ribs. Goebel has given a full account of the form-conditions in this genus.

The germinated embryos show a close resemblance to those of the climbing Cerei. In *P. anguliger* and *P. Ackermanni* (Figs. 18, 19), however, the cotyledons are somewhat less leaf-like than in *P. phyllanthoides grandiflorus* and *P. Phyllanthus* (Figs. 20, 21). In the latter the cotyledons are even more leaf-like than in any of the climbing Cerei, and show distinctly a yet greater departure from the triangular towards a rounded outline. This enlargement and rounding is, of course, in adaptation to the more mesophytic conditions which surround them. They are all of a clear green colour.

The epicotyls of the different species I have studied show considerable differences. Thus in *P. anguliger* four or five ribs form nearly at once, showing clearly enough the nearness of this species to *Cereus*; indeed the early stages of the seedlings are very *Cereus*-like. The same is true of *P. phyllanthoides grandiflorus*, and, according to Goebel's figure (Plate II, Fig. 6), of *P. latifrons*. But I think Goebel's figure does not represent this species, but some *Cereus*. *P. latifrons* has very flat broad phyllocladia and belongs near *stenopetalus*; indeed Schumann considers it probable that *latifrons* and *stenopetalus* are identical. But Goebel's own figure for *stenopetalus* (p. 104) is entirely different, and exactly what is to be expected for both *stenopetalus* and *latifrons*. *P. Phyllanthus*, which has the most leaf-like cotyledons of all, has an epicotyl of special interest (Fig. 21). The two first leaves form at right angles to the cotyledons, and the next two over the cotyledons; but the fifth comes over the third or fourth, not over the first or second, so that the epicotyl becomes at once two-ribbed and flat, and this flattening is in the plane

of the cotyledons, at right angles to their faces, and not in the plane of the faces as one would expect, and as is the case with the flattening in the Platopuntiae. The epicotyl is thus formed also in *P. stenopetalus*, as shown by the figures given by Goebel and by Lubbock. In comparing the species showing this early flattening of the epicotyl with those which have the latter ribbed, it is plain that the flat epicotyl accompanies the flattest and most leaf-like phyllocladia, and the ribbed epicotyl accompanies those less leaf-like and which oftenest show reversions towards *Cereus* in the production of joints possessing three or more ribs. The direction of the flattening in the epicotyl implies that the flattening has worked back from the adult stages, and not up from the embryonic stages, for in the latter case it would have come about in the plane of least resistance, i. e. in the plane of the faces of the cotyledons. Probably the direction is connected with the vertical position of the phyllocladia of the adults; as they are there flattened in the plane of the subtending leaf and the stem, so the flattening has worked back to stand in the plane of the two subtending cotyledons. In *P. Phyllanthus* the flattening has gone so far that the only trace of other ribs are the first and second axillary clusters. In the different species of this genus, then, we have another good illustration of the working of the principle of repetition of phylogeny in ontogeny, and a particularly good illustration of its real meaning, i. e. that a new character is acquired by adaptation in the later or adult stages of the plant, and then, in successive generations, appears earlier and earlier, until finally it has worked back to the earliest stages of the seedling, thus replacing the ancestral characters which finally cease to be repeated.

5. Genus **Epiphyllum**.

E. truncatum, Haw. Figure 55, p. 103, in Goebel; also (under name *E. Altensteinii*) in Pfeiffer and Otto, Figs. 2–5, Pl. XXVIII.

This genus, according to Schumann's monograph, contains properly but one species, the others often placed in it

belonging to *Phyllocactus*. It merges with the latter genus through such jointed forms as *P. Russellianus* and *Gartneri*. *E. truncatum* is an epiphytic, hanging, much-jointed form, too well known to need description.

None of my many seeds of *Epiphyllum* have germinated, and hence I have no data as to the embryos and seedlings except the two figures cited above. Both figures show a rather stout embryo with the hypocotyl angular, or quadrangular, in section, and with short, tapering, pointed cotyledons. This is not at all the type of embryo which one would expect theoretically in this genus. Considering the marked mesophytic character of the adults reached through the increasingly mesophytic climbing Cerei and Phyllocacti, one would expect a still further development of surface in the most mesophytic *Epiphyllum*. That this is not the case remains to be explained. Possibly we are in error as to the affinities of this genus; there is something about the cotyledons, as shown in the figures, which suggests *Rhipsalis*. But it is more likely that the extremely epiphytic habit of the species has something to do with it. The epiphytic habit always requires some xerophytic characters, and of these one of the first is reduction in size of leaf-surfaces. In this connexion the embryos of *P. Russellianus* and *Gartneri* would be of great interest.

The epicotyl in *E. truncatum*, as described by Goebel, is flat. It forms at first four polsters as the beginning of four ribs, but two of these fail to develop farther, so that the other two form a flat structure precisely as has been described and figured above for *Phyllocactus Phyllanthus*. This form of epicotyl is precisely that which we would theoretically expect.

6. Genus Pilocereus.

P. Houlletii, Lem. Figure 22

This genus merges with the large columnar Cerei, from which it is distinguished by very minor characters.

The germinated embryos of *P. Houlletii* (Fig. 22) resemble

the columnar Cerei in their broad cotyledons, though these are less broad than in the Cerei, and in the very succulent hypocotyl, which is somewhat more elongated in this form. The epicotyl has eight ribs from the start, showing how old is the rib-forming habit.

7. Genus **Cephalocereus.**

C. senilis, Pfeiff. Figure 23.

This genus merges with the last and with the columnar Cerei. In *C. senilis* (Fig. 23) the cotyledons are narrower than in the columnar Cerei, and the hypocotyl is relatively more succulent. The large size of the embryos is noticeable.

8. Genus **Echinocereus.**

E. caespitosus, Engelm. Figure 11 *g* in Engelmann, Pl. XLIV.
E. pectinatus rigidissimus, Engelm. Figure 24.
E. phoeniceus, Lem. Figure 25.
E. Engelmanni, Lem. Figure 26.
E. procumbens, Lem. Figure 27.
E. tuberosus, Poselger. Figure 28.

This genus is very closely related to *Cereus*, though Schumann now considers it as well marked. It belongs nearest to the columnar Cerei, but to a division of the latter containing small forms. All forms in this genus are small and weakly armed. According to Schumann there are four subgroups. One of these, including the erect forms and nearest to *Cereus*, includes *E. pectinatus rigidissimus*, *phoeniceus*, and *Engelmanni* (Figs. 24, 25, 26), which I have studied. The very broad cotyledons of *C. pectinatus rigidissimus* show the closest resemblance to the columnar Cerei, though the embryos are of much smaller size than in the *giganteus* section. Of much larger size, and with the cotyledons somewhat less broad, is *E. phoeniceus* (Fig. 25), but it has the distinctive parallelism as to their bases so characteristic of *Cereus*. *E. Engelmanni* (Fig. 26) has the cotyledons much reduced and resembles more nearly some of the *Echinocacti*, and there is possibly some error in my materials. A second division of this genus

includes the prostrate forms of which *E. procumbens* is a good type. The embryos of this species (Fig. 27) show a reduction in the cotyledons and in size, though they still appear *Cereus*-like. Of the third division I have no material: but in the fourth, slender creeping forms, *E. tuberosus* is the type, but its embryos resemble very closely those of the last-mentioned species.

The development of the epicotyl in the few species in which I have followed it shows nothing remarkable, for the adults in this genus share the morphological simplicity of *Cereus*. The leaves at first show a tendency to fall into spirals, and the ribs appear relatively late.

9. Genus Echinopsis.

E. Zuccariniana, Pfeiff. and Otto. Figure 29.
E. Eyriesii, Pfeiff. Figure 30.
E. multiplex, Pfeiff. and Otto. Figure in Pfeiffer and Otto, IV.

This genus is very close to the columnar Cerei, and really represents very short, many-ribbed forms of that genus. The germinated embryos as shown in *E. Zuccariniana* (Fig. 29) and *E. Eyriesii* (Fig. 30) possess remarkably slender cotyledons and an almost globular hypocotyl. The former character suggests some relationship with *Echinocactus*; and indeed Schumann in his recent monograph says, 'Die Gattung steht in der Mitte zwischen Cereus und Echinocactus.' The adult forms, however, certainly resemble the former much more closely than the latter genus, and it is probable that the connexion with *Echinocactus* is not closer than would result from a separation of *Echinopsis* from *Cereus* near where *Echinocactus* comes off from *Cereus*. In this connexion the embryos of *E. cinnabarina*, which Schumann considers as much *Echinocactus* as *Echinopsis*, would be of great interest.

The epicotyl, as is common in the *Cereus*-like forms, shows nothing of especial interest. In *E. multiplex* eight ribs form at once without any stage with spirally arranged axillary clusters, showing how old and thoroughly fixed is the rib-producing character in this genus.

10. Genus **Rhipsalis.**

R. Cassytha, Gaertn. Figure 31; also in Irmisch, Botanische Zeitung, 1876; also Pfeiffer, Fig. 13, Pl. XV.

R. (*Lepismium*) *commune*, Pfeiff. Figure in Goebel, Pl. II, Figs. 3 and 4 (misnamed *L. radicans* in explanations; see p. 102 of text); also Pfeiffer, Pl. XVI, Fig. 12.

This very important genus has been fully discussed in a well-known memoir by Vöchting [1] (which curiously neglects the early stages), and more briefly by Goebel. All of the species are small and epiphytic, with joints sometimes ribbed, sometimes cylindrical, sometimes flat. They are, without doubt, derived from slender climbing Cerei. I have been able to study embryos only of the well-known *R. Cassytha*, and figures of no others are in the books, but Goebel has given a very satisfactory account of five other species which he has studied (pp. 102, 103). *R. Cassytha* in its embryo and seedling stages has been exhaustively described and figured by Irmisch in his 'Ueber die Keimpflanzen von *Rhipsalis Cassytha*.'

In *R. Cassytha* the cotyledons are as broad as the hypocotyl, thus recalling *Cereus*, but they are stouter than in that genus and more nearly round than flat in section, and merge more evenly into the hypocotyl. The latter is also proportionally longer than in *Cereus* (Fig. 31). They are clear green in colour. From the epiphytic, and therefore to some extent mesophytic, habit of this genus, one would expect more leafy cotyledons; but perhaps, as suggested for *Epiphyllum*, the xerophytic outweigh the mesophytic influences in the epiphytic life. The figure given by Pfeiffer suggests very much more leaf-like cotyledons than do Goebel's figures, and it is difficult to believe they represent the same species.

In *R. Cassytha* the epicotyl contains at first four distinct ribs, which merge above into the spirally-placed clusters characteristic of this species. In this we have another good illustration of biogenesis; the ribs are no doubt an inheritance

[1] In Pringsheim's Jahrbücher, IX.

from *Cereus*, while the spiral arrangement in the adults is a re-development of what is really a primitive character in the family Cactaceae. Goebel shows that several other species of this genus form the beginnings of four ribs: some continue to form them for a time, but *R. commune*, which when adult is triangular, immediately drops one and becomes triangular; while in *R. rhombea* and *R. pachyptera*, which in the adults have very flat, thin phyllocladia, two of the ribs are represented by single polsters only, and the epicotyl from the first is flat in the plane of the cotyledons in precisely the same way as has been described and figured above for *Phyllocactus Phyllanthus*. The exactly parallel character of the mode of formation of the epicotyl in *Phyllocactus* and *Rhipsalis* accompanies a parallelism in the adults in these two genera. This does not in the least mean that these groups have had any direct genetic connexion; probably both have come off from climbing Cerei but quite independently, *Phyllocactus* from *triangularis*-like, but *Rhipsalis* from very slender *flagelliformis*-like forms. The parallelism, then, has come about through modifications due to similar habit working upon a similar morphological basis. In both genera the ribbed character is primitive, and the flattened condition newly acquired, and the working back of the latter into the seedling until finally it quite obliterates the ribbed condition affords some of the best examples and illustrations of the true meaning of repetition of phylogeny in ontogeny yet adduced among plants.

11. Genus **Hariota**.

I have no data, nor does the literature contain any, upon the embryology of this group.

12. Genus **Pfeiffera**.

P. cereiformis, Salm-Dyck. Figure, Pl. IX of Pfeiffer and Otto; also in Goebel, p. 86.

I have not myself studied this genus. The figure given by Goebel shows a short stout epicotyl with stout pointed

cotyledons, in which one can imagine a resemblance to those of *R. Cassytha*, and which also recall *Cereus*. The remarkable figure given by Pfeiffer I do not understand, and think it must represent something abnormal, for the epicotyl as he figures it is like nothing I have seen in this family. Probably the embryos and seedlings would show whether this genus belongs more nearly with *Rhipsalis* or with *Cereus*. Vöchting, reasoning from anatomy, considered that *Pfeiffera* is the nearest living representative of the connecting forms between *Cereus* and *Rhipsalis*.

13. Genus **Echinocactus**.

E. robustus, Otto. Figure 6 in Pfeiffer, Pl. XVI.
E. Wislizeni, Engelm. Figure 32.
E. viridescens, Nutt. Figure 33; also in Lubbock, II, p. 8.
E. texensis, Hopf. Figure 34.
E. cornigerus, DC. Figure 36; also in De Candolle (Mémoire), Pl. X, Figs. 5–7.
E. longihamatus, Gal. Figure 35.
E. setispinus, Engelm. and Gray. Figure in Maxwell, p. 29.
E. recurvus, Link and Otto. Figure in Pfeiffer (as *E. spiralis*), Pl. XVI, Fig. 7.
E. ingens, Zucc. Figure 37; also in Pfeiffer, Pl. XVI, Fig. 5; also in Goebel (as *E. aulacoganus*), p. 86.
E. horizontalonius, Lem. Figure 38.
E. phyllacanthus, Mart. Figure in Zuccarini, Pl. II, Fig. 3 (seedling).
E. Williamsii, Lem. Figure 39.
E. myriostigma, Salm-Dyck. Figure 40.
E. capricornis, A. Dietr. Figure 41.
E. lophothele, Salm-Dyck. Figure 42.
E. Scheerii, Salm-Dyck. Figure 43.
E. Simsoni, Engelm. Figure in Engelmann, Simson's Expedition, Pl. II, Fig. 16.
E. corynodes, Otto. Figure in Zuccarini, Pl. I, Fig. III, 3, 4; also Pl. II, 2.

This very large genus contains condensed globular (becoming very short cylindrical), strongly ribbed forms, with the ribs rarely broken to tubercles, giving a transition to *Mamillaria*, and all of true desert habitat. They doubtless have come off from the columnar Cerei low down on the stem, and near *Echinopsis*.

Of the various sub-genera, some of which have been considered in the past as distinct, the one which comes nearest to the Cerei is, no doubt, Schumann's *Euechinocactus*. In

this division I have had no seeds; but *E. robustus*, of which figures of embryos are given by Pfeiffer, belongs here[1]. Though in these figures the breadth of the cotyledons cannot be seen, the whole aspect, particularly of his figure *c*, is very *Cereus*-like, thus confirming the primitive position of this sub-genus.

Most important, and typical of this genus, is Schumann's division of *Ancistrocactus*. In it I have studied embryos of *E. Wislizeni* (Fig. 32), *viridescens* (Fig. 33), *texensis* (Fig. 34), *longihamatus* (Fig. 35), *cornigerus* (Fig. 36), while a figure of *E. recurvus* is given by Pfeiffer, and of *setispinus* by Maxwell. It will be noted that all of these figures agree in showing a much swollen hypocotyl with thick, pointed, flat-faced cotyledons which are much less broad than the hypocotyl, and do not merge gradually into it all around, but on the sides and faces are separated by a sharp angle which is at times almost a slight constriction. The cotyledons are smallest in *E. cornigerus*. The form of cotyledons shown in this group seems to be as characteristic for the genus *Echinocactus* as the broad and flatter ones are for *Cereus*, and the constancy of form through this large sub-group implies marked constancy in this character, and hence reliability as a test for affinities. In this group I have followed the development of the epicotyl in *E. Wislizeni*, *texensis*, and a few others. The axillary clusters are at first distinct, but stand in lines, and very soon run together to form ribs. In *E. texensis* new ribs appear among the older ones, starting abruptly with a small piece of rib below a spine-cluster. This occurs also in other species, and shows that in these forms rib-formation has become so fixed that the rib is itself a morphological element. They, together with most

[1] Of this species he figures three very different forms, and draws conclusions therefrom that the form of embryos and seedlings in the same species is very variable. I have only to say that I have never seen in any one of the many species of Cactaceae I have studied any such variation as Pfeiffer figures and describes, aside of course from specimens injured in some way or drawn for want of light. Seeds are apt to mix in the pots, as I have pointed out, and some of his different forms may be different kinds.

other forms of this genus, show the earlier formed spines feathered with fine hairs, which are prolongations of epidermal cells of the spines. This feathering does not occur in *Opuntia*, and not commonly in *Cereus*, or in any of the groups closely related to them, though it occurs also in *Mamillaria* and its relatives. In *E. Beguin*, in the young seedlings, it is particularly well marked, and it is this character intensified which gives us the plumose spines of *Mamillaria Bocasana*. Possibly when well developed it has an ecological use, but otherwise I think it may represent one of those lines of spontaneous variation which have no regard to utility. The subsequent and adult development of the species in this genus is simple, and very closely like that of the columnar Cerei.

Schumann, in his monograph, has placed *E. Scheerii* in this division of *Ancistrocactus*, but its embryology places it with the division *Thelocactus*, under which I shall consider it, and where indeed Schumann, in his Cactaceae in Engler and Prantl, originally placed it.

Another division of much importance is Schumann's *Cephalocactus*, in which I have studied *E. ingens* (Fig. 37) and *horizontalonius* (Fig. 38). These two species show embryos very like one another, but very different from those of the division last studied. In both the hypocotyl is very stout and somewhat elongated, and in both it shows a slight flattening, which is perhaps the result of their position in the seed. But the chief peculiarity is in the cotyledons, which, so far from being pointed, are rounded and scarcely raised out of the hypocotyl, and this character is more extreme in *E. horizontalonius* than in *E. ingens*. In fact the latter species, except for its large size, suggests *Anhalonium*, presently to be considered. The early development of the epicotyl in *E. horizontalonius* is shown in Fig. 38 *d*, where the characteristic rounded tubercles are of very unusual form, and the early development of *E. ingens* is much like it, though the tubercles are less rounded and pass at once to ribs. An excellent figure of a young plant at a much later stage is given by Engelmann (Pl. XXXII).

Very closely related to *Cephalocactus* is *Lophophora*, sometimes made a distinct group. It contains the species *E. Williamsii*. The embryos (Fig. 39) are nearly globular, and have very small rounded cotyledons, in this character resembling those of *E. horizontalonius*. The epicotyl in *E. Williamsii* bears rounded tubercles like those figured (Fig. 38 *d*) for *E. horizontalonius*, though less prominent, and bears small feathered spines. The spines in the adults, though seeming to be absent, are really present in this species, as I have elsewhere shown[1], and the species really represents a form like a young *horizontalonius* (compare Engelmann's figure) without the large spines, and there can be no doubt that it is an *Echinocactus*, closely related to the division *Cephalocactus*.

Another division often given distinct generic rank is *Astrophytum*, of which I have studied embryos of *A. myriostigma* and *A. capricornis* (Figs. 40, 41). Embryos of these two are much alike, and very different from those of any others I have studied. The hypocotyl is swollen, but more on one side than the other, which is due to the position in the seed; and the root, for the same reason, is somewhat on one side. The cotyledons are rather narrow but pointed, though less so than in some other divisions. In the epicotyl the first clusters at right angles to the cotyledons are very prominent, marking the immediate beginning of the ribs, and making the plant, as seen from above, almost quadrangular. The ribs hence develop earlier in this species than in any other I have seen in the family. Two others form over the cotyledons, and these four form the starting-point for four strong ribs which, by the forking of one, soon become five, which distribute themselves over the circumference. In their later growth these two species become less alike. The clusters of characteristic remarkably pitted hairs occur upon young seedlings. The spines in the first-formed clusters are well-developed, but later clusters have them very small.

In the important division *Stenocactus* I have had no

[1] Flora, op. cit., p. 72.

material, nor do I understand the curious figure given for *E. phyllacanthus* by Zuccarini.

In *Malacocarpus* I know only the figure of *E. corynodes* given by Zuccarini. The embryo has very small cotyledons, which are, however, prominent and pointed, on a nearly globular hypocotyl.

In the important *Thelocactus* I have studied embryos of *E. lophothele* and *E. Scheerii* (Figs. 42, 43). Both have nearly globular hypocotyls, with very small rounded cotyledons, and in these respects approach very near to *Mamillaria*, which genus indeed these species, particularly *E. Scheerii*, approach very closely, as is shown by other considerations given in the systematic works. *E. brevihamatus* would be an interesting form to study in this connexion. It is well known that *E. Scheerii* shows clearly the furrow on the upper side of the tubercle which represents the drawing out of the vegetative point by intercalary growth of the tubercle in that region, and this feature becomes of great importance in *Mamillaria*. The earlier tubercles do not show it, but in the development of the epicotyl in *E. Scheerii* it is easy to see how this groove develops ontogenetically. The earlier tubercles show no trace of it, but after some twenty of them are formed, one appears with a tiny groove projecting slightly from the spine-cluster on the upper side of the tubercle; the next shows this a little longer, and the next yet longer, and so on until the groove is complete. But it is not until much older tubercles than these are formed that the flowers first appear.

The fine figure of *E. Simpsoni* given by Engelmann shows pointed and rather large cotyledons, and seems related more nearly to the *Ancistrocactus* group, though its replacement of ribs by tubercles would suggest that it belongs to the division *Thelocactus*. The melting away of ribs into tubercles, however, is a character which can readily reappear again and again, and need not indicate relationship; and the close relationship of this species, emphasized by Engelmann, to *E. intertextus*, a ribbed species, confirms this.

(Genus **Astrophytum.** Treated under Echinocactus.)

(Genus **Malacocarpus.** Treated under Echinocactus.)

14. Genus **Melocactus.**

M. communis, Link and Otto. Figure in De Candolle, Revue, Pl. VI. Also (as *Cactus melocactus*) in De Candolle, Organographie, Pl. XLVIII, Fig. 3.
M. amoenus, Hoffmgg. Figure in Zuccarini.
M rubens, Pfeiff. Figure in Pfeiffer, Pl. XVI, Fig. 9.
M. Lehmanni, Miq. Figure in Miquel, Monographia, Pl. II.

This genus includes nearly globular strongly-ribbed forms especially characterized by a strong cephalium. I have had no seeds, and the figures above cited are hardly detailed enough to allow conclusions to be drawn from them. All show a globular, or nearly globular, hypocotyl. *M. amoenus* in Zuccarini's figure shows large thick pointed cotyledons, recalling the *Ancistrocactus* division; while *M. rubens*, as given by Pfeiffer, suggests rather *Cephalocactus*, an altogether unlikely difference between these two; while *M. Lehmanni*, in Miquel's picture, is different from the other two. De Candolle's figure of *M. communis* is of interest in showing below the huge swollen stem, two projections which he mistook for cotyledons, but which, as Miquel[1] pointed out, are not so, but were probably two rootlets in that position. The nearly globular structure which he mistook for plumule is combined hypocotyl and epicotyl, with the cotyledons invisible. The presence of a lateral root in that position in Miquel's figure of *M. Lehmanni* confirms this. The beginning of growth of the epicotyl shown by Miquel is insufficient to give a clear idea of it.

15. Genus **Leuchtenbergia.**

L. principis, Fisch. Figure 44.

In this genus is but a single species, a rare and remarkable plant with very long, almost leaf-like, tubercles bearing papery

[1] Ann. Sci. Nat., Bot., 2nd Ser., XIV, 62, 63.

spines and flowers at their summits. The germination and early stages have not been described. I have obtained seeds from Mr. William Tell, of Austin, Texas, who obtained them from a correspondent in Mexico; and as living plants of this species have come from the same source, the identification is to be trusted. The embryo (Fig. 44) is altogether *Echinocactus*-like, with the pointed cotyledons so characteristic of that genus. The hypocotyl is deep red in colour though the first leaves of the epicotyl are white, an unusual feature. The epicotyl develops first two leaves at right angles to the cotyledons, which bear spine-clusters in their axils, and both leaves and clusters are soon carried up on the tops of strongly developed cylindrical tubercles, which, except for those of *Anhalonium*, are larger than those of any Cactaceae I have seen, and this fact is in itself confirmation of the identity of my seeds as of this species. A third tubercle then develops at right angles to the first pair, and later a fourth. The spines are also *Echinocactus*-like, much feathered with hairs, and very different from those of the adult plants, and this feature persists into seedlings of considerable size, as I have seen in a specimen formerly described by me[1]. At this writing my specimens have not advanced beyond the stage shown in the figure 44 *c*, but I hope to obtain from this material the full life-history of this most remarkable of the Cactaceae. But all features show its close connexion with *Echinocactus*, though I cannot say with which division. The nearest approach to the remarkable tubercles of the epicotyl that I have seen is in *E. bicolor*, which has large spirally-arranged tubercles, though they are not so large as in *Leuckenbergia*.

16. Genus **Mamillaria**.

M. elephantidens, Lem. Figure 45.
M. latimamma, DC. Figure in Goebel, p. 86.
M. radiosa neo-mexicana, Engelm. Figure 46.
M. centricirrha, Lem. Figure in Schumann, in Engler and Prantl, p. 170.

[1] Flora, op. cit., p. 74.

M. Semperviri, DC. Figure 47.
M. Goodridgii, Scheer. Figure 48.
M. glochidiata, Mart. Figure in Zuccarini, Pl. I, Fig. I, 4.
M. tentaculata, Link and Otto. Figure in Pfeiffer, Pl. XVI, Fig. 8.

This very large genus contains the smallest species of the family, and consists of globular to short cylindrical forms with spirally-arranged tubercles (and never ribs), with spine-clusters at the ends of the tubercles and flowers in their axils. In one division the tubercles have a longitudinal furrow along the upper face, and these connect with the furrowed Echinocacti of the division *Thelocactus*. In the other division the furrow is wanting. The explanation of its presence in the one group and its absence in the other I have elsewhere given [1].

Throughout this genus the embryos show a very succulent and usually nearly globular hypocotyl, with cotyledons always small, sometimes indeed so small as to have been considered wanting. They are usually rounded, but sometimes more or less pointed.

In the division with the furrow I have had seeds only of *M. elephantidens*, which have produced an embryo of remarkably large size (Fig. 45), with an elongated hypocotyl and rather broad rounded cotyledons, suggesting the *Cephalocactus* division of *Echinocactus*, with which however it is scarcely at all possible that *Mamillaria* has directly any affinities. Since Goebel's figure of *M. latimamma* shows the same elongated hypocotyl, it is possible this is a common characteristic of this division. In this connexion the embryos and seeds of *M. macromeris* would be of especial interest. If this furrowed division of *Mamillaria* is derived, as other considerations seem to show that it is, from the *Thelocactus* division of *Echinocactus*, then the large size and elongated hypocotyl must be re-acquired and not primitive, for neither character appears in *E. Scheerii* (Fig. 43), a good representative of *Thelocactus*. The epicotyl in *M. elephantidens* develops the clusters spirally on short tubercles, with no

[1] In Flora, op. cit., p. 75.

trace of rib-formation. I have not observed the beginning of the splitting of the vegetative points on the tubercles, but no doubt it comes about there precisely as in *E. Scheerii.*

M. radiosa neo-mexicana belongs also with the grooved forms; its cotyledons (Fig. 46) are pointed, a feature very rare in this genus, but their small size would prevent any confusion of this with a small *Echinocactus.*

In the furrowless division occur the smallest embryos, and of the several species I have studied all are much alike. As shown by *M. Goodridgii* and *Sempervivi* the hypocotyl is very swollen, larger above the middle and with small rounded cotyledons. In the development of the epicotyl in this division I have not yet determined the very important point of the exact mode of appearance of the vegetative point which in the adults stands in the axils of the tubercles and produces the flowers. Theoretically this ought to appear as it does in the furrowed Echinocacti and furrowed Mamillariae, i. e. the point which on the first formed tubercles is single should on some of the later become stretched out, and in still later ones the stretching should go yet farther so as to separate it into two parts, and so on, the stretching taking place at earlier and earlier stages in the growth of the mamilla until finally it occurs before this has become raised from the main stem.

17. Genus **Anhalonium.**

A. fissuratum, Engelm. Figure 49; also in Goebel, Pl. II, Fig. 7
A. prismaticum, Lem. Figure 50.

This small, but morphologically very important, genus contains only small somewhat *Mamillaria*-like forms with angular tubercles and no visible spines. They are flattened above and in shape like a top with the greater part buried in the earth. The tubercles show two types, one with a furrow and one without, in this showing parallelism with *Mamillaria.*

Important though this genus is, but a single good figure

of its embryos or seedlings has been published, that given by Goebel, cited above. In the embryos of both *A. fissuratum* and *A. prismaticum* which I have studied, the hypocotyls are very succulent and somewhat elongated. The cotyledons are hemispherical, merging without visible boundary into the hypocotyl, rather broad, so that the groove between them is much longer than in any of the Mamillariae I have studied (compare Fig. 49*c* and 50*b* with 47*c* and 48*c*), and in this respect, as well as in the way in which they merge without break into the hypocotyl, they are much more like *E. horizontalonius* (Fig. 38) than like any *Mamillaria*. The rather abrupt ending of the groove and its slight broadening there is also characteristic of both *Anhalonium* and *E. horizontalonius*, and it is not marked in any *Mamillaria* I have seen. This fact however can hardly mean relationship, but merely coincidence, for the two vegetative points to the tubercle show a close relationship with *Mamillaria* and an origin from *Thelocactus*. I think it most likely that *Anhalonium* has come off from *Thelocactus* independently of, but near to, *Mamillaria*.

The development of the epicotyl in both species is remarkable. The first tubercles are long and slender (Fig. 49*d* and 50*c*; also Goebel, Plate II, Fig. 7), and bear several feathered spines at their tips. The whole appearance of these tubercles strongly recalls that of the adult tubercles of *Leuchtenbergia*, and it is very difficult indeed to resist the belief that they are in some way connected. But the markedly pointed cotyledons of *Leuchtenbergia* in contrast with the very low rounded ones in *Anhalonium* makes any direct relationship well-nigh impossible, and we must attribute the resemblance of the tubercles to parallelism and not to any direct connexion. On the other hand, following the hint given by the cotyledons, it is not at all difficult to imagine the pronounced tubercles of a form like *E. horizontalonius* (Fig. 38*d*) growing out into the tubercles of *Anhalonium*. As the epicotyl becomes older the new tubercles become broader and flatter, and pass gradually over into the well-known angular tubercles of the adults.

I have not yet been able to determine the important point of the mode of formation of the inner vegetative point, but have no doubt it comes about as in *Thelocacti* and *Mamillaria*. It should be particularly plain in *A. fissuratum*.

A feature which is prominent in this genus and not found in any other Cactaceae, so far as I know, is the early growth in succulence of the root along with the hypocotyl. The root, shortly after the embryo is out of the seed, instead of remaining small, begins to swell not only as fast as, but often faster than, the hypocotyl. For a time a constriction marks the boundary between the two (shown in Fig. 50*c*), but later this disappears, and in the adult plant the true epicotyledonary stem is extremely short, while the great visible part below the tubercles is really root, as Goebel has already surmised. This unusual succulence of the root is no doubt correlated with the half-buried habit of the plant. Succulent roots of another kind occur also in *Cereus tuberosus*, *Echinocactus napinus*, and perhaps other species.

18. Genus **Pelecyphora.**

This genus, with its single odd species, is but slightly removed from *Mamillaria*, with which it is no doubt connected through a form like *M. micromeris*, which somewhat closely approaches it in many characters. From my seeds several embryos were produced which, however, did not differ from those of the smaller kinds of *Mamillaria*. The cotyledons were very minute on a nearly globular hypocotyl.

Summary of the Form-factors of Embryos and Seedlings.

We may now group together the facts just described and note to what conclusions they point. Taking first the germinated embryos, it is plain that as a whole there is, from *Pereskia* through *Cereus* and *Echinocactus* to *Mamillaria*, a progressive condensation in bulk, and diminution of surface

brought about by the increasing approach to a spherical form of the hypocotyl, and diminution of the cotyledons. The only marked exception to this is found in the Platopuntiae, climbing Cerei, Phyllocacti, and *Rhipsalis* ; but in these cases, as I have already pointed out, there is in common a return to a more mesophytic habit, which amply explains the larger spread of surface and consequent relative increase of cotyledon and diminution of hypocotyl. This progressive condensation of the embryos, however, runs strictly parallel to the condensation in the adults, and in both cases is least in *Pereskia*, more in *Opuntia*, yet more in *Cereus*, still more in *Echinocactus*, and most of all—indeed reaching near to its utmost possible theoretical limit—in the nearly globular forms in *Mamillaria*. In the adults this condensation is due to adaptation to a more and more desert habitat. There is however this important difference to be noted between the two series, that in the progressive condensation the embryos lag behind the adults, and this is plainest in the diminution of the cotyledons. Thus in *Opuntia* the leaves are very evident in both adults and embryos, but even here the cotyledons have diminished from those of *Pereskia* less than have the leaves of *Opuntia* diminished from those of *Pereskia*; in *Cereus* this is yet more evident, for here the cotyledons are still large and broad, while the leaves of the adults are reduced to small scales and often are but tiny rudiments. In *Echinocactus* the cotyledons are still somewhat prominent, but the leaves of the adults are represented only as microscopic rudiments soon merging into the stem in later growth, while in *Mamillaria* the leaves are as in *Echinocactus*, and the cotyledons themselves are nearing the vanishing-point. It is true there are some seeming exceptions to this rule, as in the *Cephalocactus* division of *Echinocactus*, but in reality, as this is one of the most condensed of the divisions of the genus, it illustrates and does not contradict the rule. Indeed, as far as my material allows of a judgment, it seems plain that the same principle applies within the limits of each genus, i. e. the more condensed the adults, the more succulent the hypocotyl

and the less developed the cotyledons, and the lagging of the embryos behind the adults is probably true here also. The latter principle may be found true where a single species of a given genus suddenly deviates from the normal for that genus. This lagging behind amply explains why adult and embryo do not always correspond. But however it may be in details, I think there is no doubt that the ground-form of the embryos is imposed by the ground-form of the adults, though not so much by the immediately preceding as by the more remotely preceding ancestors.

When we turn to consider the dynamics of the connexion between adults and embryos, exactly how it is that the former can influence the latter, we face a difficult question. At a first glance one might explain it as due to the fact that as embryos and adults live in the same places, and hence under much the same conditions of dryness; and since condensation or spread of surface is chiefly determined by dryness of the habitat, therefore the spread of surface must correspond in adults and embryos, i. e. through independent adaptation of each to the same external conditions. But only a little observation is needed to disprove this: for on the one hand the germinated embryos live under very different external conditions from the adults, in that they are growing only at the time of the yearly rains; and on the other, *Cereus*, *Echinocactus*, *Opuntia*, and *Mamillaria* embryos grow side by side on the same desert and thrive equally well, though of such extremely different forms. Direct adaptation therefore to the surroundings, except in the case of those with a re-acquired mesophytic habit as *Phyllocactus*, can have but little to do with the form of the embryos. Again, one may suppose that the shape of the seed determines that of the embryo; but this simply replaces the difficult question at issue by the very much more difficult one of how the adult plant is able to influence the form of its seeds so that these will mould an embryo answering in form to itself. Moreover the relative size of cotyledons and hypocotyl is not in the least degree affected by the shape of the seed. In some minor particulars however,

I think the seed does influence the form of the embryo, as for example in the difference of size between the two cotyledons in many species, particularly Platopuntiae. Observation shows that the smaller is on the concave side, covered by the convex larger one. Again, possibly the forking of the tip of one cotyledon in *Cereus Bonplandi* may be due to this, and certainly the one-sidedness of the root in *E. myriostigma* and *E. capricornis*, and the slight asymmetry in both planes noticed in nearly all cotyledons and hypocotyls, including the slight pointing to one side of the pointed cotyledons in *Cereus* and *Echinocactus*. But such are very minor effects.

Of course in any given generation the form of the embryo, aside from slight irritable responses to light, &c., is determined by heredity. But heredity is but the sum and resultant of past experiences, and hence in the present case is largely a study of the facts of past environments. This suggests an explanation which I believe to be the true one, i.e. that the form of the adults, like any other character, once acquired—it matters not for our present purpose how—as it becomes more and more fixed and intensified, tends to work back into earlier and earlier stages in the ontogeny of the successive individuals; until finally, a character adaptively acquired by the adults works back into the epicotyl, of which I have shown many cases in this paper, and finally into the embryo itself. But while the working back into the epicotyl may be comparatively rapid, the passage into the embryo seems to be very slow, as is explained by the considerable lagging of those behind the adults, due no doubt to the fact that the embryos have a set of activities of their own in their early life which keeps them from being too plastic to other influences working upon them.

In summary then the shapes of the embryos in Cactaceae seem to be determined by three leading factors. First, the ground-form of the embryos answers in general to the ground-form of the adults, and alters with the latter by the working back of newly acquired characters. Second, there is an occasional expansion of surface, with increased cotyledons

and relatively diminished hypocotyl, allowed by exposure to more mesophytic habitat. Third, some minor details of outline are due to the position of the embryos in the seed.

The form-factors of the epicotyl are simpler than those of the embryos, and from what has been said in the preceding pages it will appear that we have in the epicotyls remarkably clear examples of the working back of the characters of the adults. The first leaves of the epicotyl (very small except in *Pereskia* and *Opuntia*), and indeed often the cotyledons themselves, produce axillary buds which at once develop the characteristic spine-clusters. This is true in all of the species that I have examined throughout this family; the very first formed axillary structures are true spines. There seems to be abundant evidence that the spines in this family really are metamorphosed leaves and not emergences or other dermal or epidermal structures; and recalling the principle of repetition of phylogeny in ontogeny, one would expect to find in these first formed axillary clusters some trace of a leaf-nature showing in the spines, which is never the case. This fact, however, is not necessarily evidence for a non-phyllome origin of the spines, since it may, and probably does, mean simply that the production of leaf-spines from axillary buds is very old, preceding the differentiation into genera, which indeed *Pereskia* proves to us is the case. It may be that this ancient habit of producing axillary spine-clusters is the oldest existent characteristic of the family, and may yet give the key to its still unknown phylogeny. The epicotyl of course tends to repeat its hereditary characters, and does so until these are obliterated by the working back of new ones, and it is the different stages of the working back of these which give us the very fine examples of repetition of phylogeny in ontogeny which appear in the climbing Cerei, Phyllocacti, *Rhipsalis*, &c. Taking the family as a whole we may picture successive waves, as it were, of characters acquired by adaptation in the adults sweeping back into the later seedlings, and wiping out earlier characters. The more superficial features of these waves do not however affect the

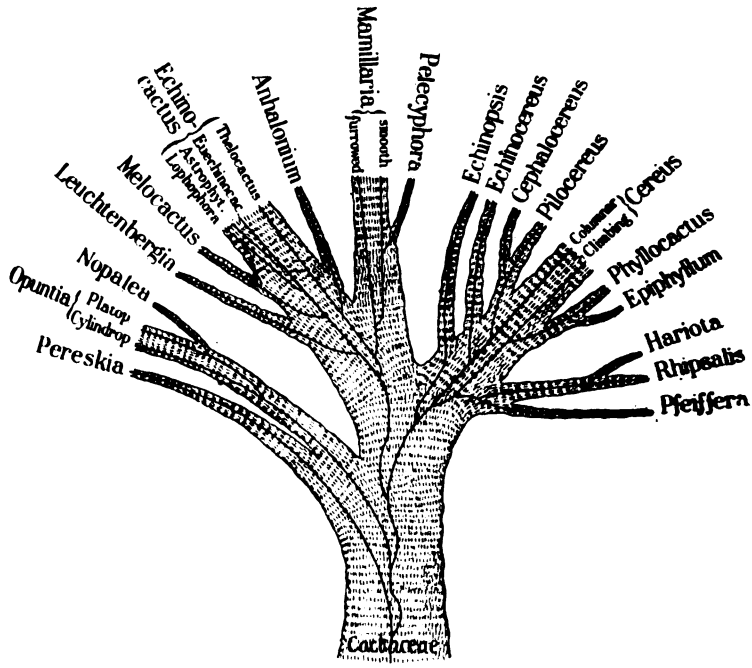
embryo, for the transition from epicotyl to embryo forms a barrier against which the crests of the waves are broken, and behind which only the influence of the ground-swell, as it were, is felt. It is the condition of these waves at this moment which gives us the present embryology of the family.

PHYLOGENY OF THE GENERA.

We may now summarize what the preceding facts indicate as to the phylogeny of the genera. Taking them all into account, together with others drawn from the comparative anatomy of the adults, and to some extent others from the floral and fruit anatomy, the relationships appear to be as expressed in the following diagram. The outline of the main trunks and branches is intended to express the appearance of the whole group as it appears to us at the present day, while the axial lines are intended to represent the probable historical relations of the different genera, their place and relative time of origin from one another.

All considerations show that *Pereskia* is nearest to the original stem-form of the family, and that *Opuntia* is derived from it, though the connecting forms still existing make of them a single trunk. From *Opuntia* came off *Cereus*, and our columnar Cerei are nearest to the primitive form as their cotyledons show, though the original Cerei must have been somewhat tubercled after the manner of *Opuntia* rather than strongly ribbed. From columnar Cerei came off in succession *Echinopsis*, *Echinocereus*, and *Pilocereus*, and from the last *Cephalocereus*. Well down in the stem of the columnar Cerei came off the climbing forms, giving another incipient genus, and from these came first of all *Pfeiffera*, with its early branch *Rhipsalis*, and later *Hariota*, and *Phyllocactus* with its branch *Epiphyllum*. From the columnar Cerei again, but low down and near *Echinopsis*, came off *Echinocactus*, which soon gave off as a branch the furrowed forms which gradually changed from the ribbed to the tubercled forms. These tubercled Echinocacti, with their furrow, gave rise to the

Mamillariae with a furrow, and these in turn by its disappearance to the smooth tubercled forms, and from these came finally the most specialized of all of the genera, *Pelecyphora*. Returning to the Echinocacti, we find *Melocactus* as a later branch, and as earlier ones *Leuchtenbergia* and *Anhalonium*. The relations of the two latter are somewhat



puzzling. Though the embryos of *Anhalonium* in some ways recall the *Cephalocactus* division of *Echinocactus*, yet the presence of two growing-points in each tubercle places it near *Mamillaria* and relates it to *Thelocactus*, and it has probably come off from the latter near to but independently of *Mamillaria*. As to *Leuchtenbergia*, the pointed cotyledons

and single growing-points to the tubercles suggest an origin from *Echinocactus* in some division other than *Thelocactus*, but at present I have no further data. It must come from near a form with the ribs replaced by tubercles.

Since my materials were chosen especially to represent the embryology of the genera and leading groups, and hence only rarely include closely related species, it does not give a fair idea of the extent to which embryology can be used in this family as a clue to the relationships of closely related or doubtful species. Obviously a large amount of accurate data and a thorough knowledge of the principles controlling form-changes must be accumulated, and the present study needs to be supplemented by a minute study of the embryology of groups of related species, and this I hope to make in the form of morphological life-histories of certain species. I have no doubt that the very stable characters of the embryos, and the remarkable way in which the epicotyls so often show the characters of the immediate ancestry, will make them of great systematic importance. And when to this we add the fact that most of the genera and many of the species in this new family are still in the nascent state, and that most of the connecting links are still in existence, I think it will be possible to recover the phylogeny of genera and species in this family to an altogether rare degree of completeness.

BIBLIOGRAPHY

DE CANDOLLE, A. P.: Organographie végétale. Paris, 1827. Vol. II.

———————— Revue de la Famille des Cactées. Mém. Mus. d'Hist. Nat. Paris, XVII, 1827.

———————— Mémoire sur quelques espèces de Cactées nouvelles ou peu connues. Paris, 1834.

DUVERNOY: Untersuchungen über Keimung, Bau und Wachsthum der Monocotylen. Stuttgart, 1854.

ENGELMANN, GEORGE: Several papers in his Collected Works. Cambridge, Mass., 1887.

GANONG, W. F.: Beiträge zur Kenntniss der Morphologie und Biologie der Cacteen. Flora, Ergänzungsband, 1894.

———— Upon Polyembryony and its Morphology in Opuntia vulgaris. Bot. Gazette, XXV, 1898.

GOEBEL, KARL: Pflanzenbiologische Schilderungen. Marburg, 1889. Vol. I.

IRMISCH, J.: Ueber die Keimpflanzen von Rhipsalis Cassytha und deren Weiterbildung. Bot. Zeit. 1876.

KLEBS, G.: Beiträge zur Morphologie und Biologie der Keimung. Untersuchungen aus dem botanischen Institut zu Tübingen, I, 1885.

LABOURET, J.: Monographie de la Famille des Cactées. Paris, 1858.

LUBBOCK, SIR JOHN: A Contribution to our Knowledge of Seedlings. London, 1892. Vol. II.

MAXWELL, C. F.: Some Morphological Relationships of the Cactaceae. Texas Academy of Science, 1894. (With MS. additions.)

MIQUEL, F. A. W.: Monographia Generis Melocacti. Verhandl. Leop.-Carol. Akad. I, XVIII.

———— Sur la Germination de Melocactus, Bull. des Sci. Phys. et Nat. en Néerlande, 1839, 47; also Ann. Sci. Nat., 2nd ser., 1840, 62.

PFEIFFER, L.: Neuere Erfahrungen über mehrere Cacteen. Nov. Acta Leop.-Carol. Akad. XIX.

PFEIFFER, L., AND OTTO, F.: Abbildung und Beschreibung blühender Cacteen. Kassel, 1843–1847.

SCHUMANN, K.: Cactaceae in Engler and Prantl, Natürliche Pflanzenfamilien, 1894.

———— Gesamtbeschreibung der Kakteen, Neudamm, 1897, 1898.

———— Cactaceae in Flora Brasiliensis, 1890.

ZUCCARINI, J. G.: Plantae novae vel minus cognitae, &c. Abhandl. der Münchener Akad. Math. Phys. II. 1831–1836.

EXPLANATION OF FIGURES IN PLATE XXVI.

Illustrating Professor Ganong's paper on the Cactaceae.

All figures drawn from nature with camera lucida. The species of *Pereskia* and *Opuntia* are natural size; all others are two and a half times natural size, unless otherwise explained. Letters *a*, *b*, *c* designate different views of the same specimen. Average specimens were always selected for drawing.

Fig. 1. *Pereskia aculeata.*

Fig. 2. *Opuntia bernardina*: *c*, cross-section of cotyledon × 5; *d*, do. of hypocotyl × 5; *e*, specimen from which buds were removed, and which had grown to double size.

Fig. 3. *O. echinocarpa*: *c*, specimen bearing a bud in the fork near the tip of a cotyledon.

Fig. 4. *O. basilaris ramosa.*

Fig. 5. *O. vulgaris*: *c*, cross-section of cotyledon × 5; *d*, do. of hypocotyl × 5.

Fig. 6. *O. Ficus-indica.*

Fig. 7. *O. Engelmanni occidentalis.*

Fig. 8. *Cereus Thurberi.*

Fig. 9. *Cereus giganteus.*

Fig. 10. *C. peruvianus.*

Fig. 11. *C. Hystrix.*

Fig. 12. *C. Bonplandi*: *c*, older specimen showing removal of axillary buds away from epicotyl.

Fig. 13. *C. Martianus.*

Fig. 14. *C. grandiflorus.*

Fig. 15. *C. nycticaulis.*

Fig. 16. *C. spinulosus.*

Fig. 17. *C. triangularis*: *c*, specimen issuing from seed to show hair-collar and basal swelling × 5; *d*, older specimen to show do. × 5.

Fig. 18. *Phyllocactus Ackermanni.*

Fig. 19. *P. anguliger.*

Fig. 20. *P. phyllanthoides grandiflorus.*

Fig. 21. *P. Phyllanthus*: *a*, *b*, younger specimen; *c*, *d*, older specimen.

Fig. 22. *Pilocereus Houlletii.*

Fig. 23. *Cephalocereus senilis.*

Fig. 24. *Echinocereus pectinatus rigidissimus.*

Fig. 25. *E. phoeniceus.*

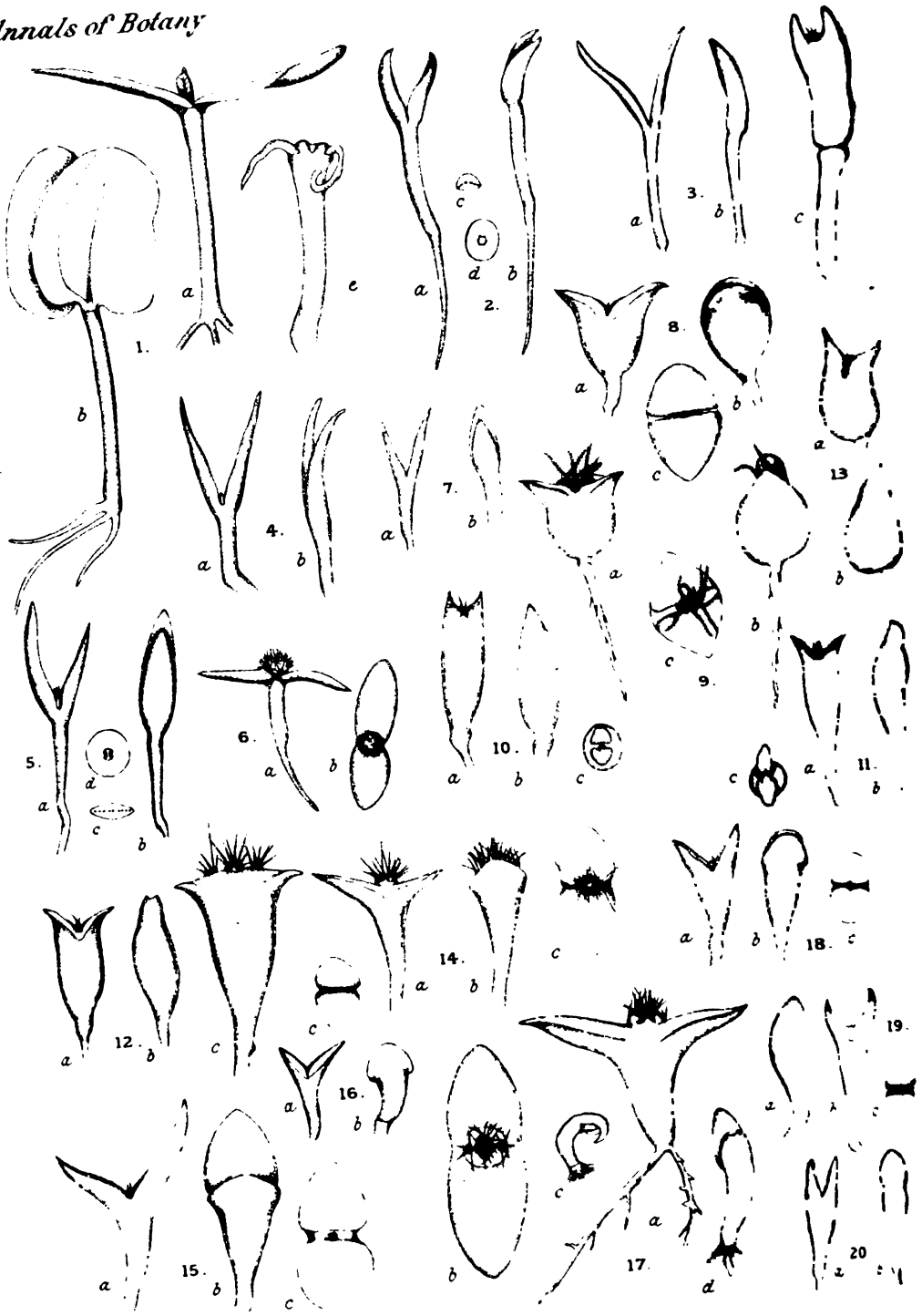
Fig. 26. *E. Engelmanni.*

Fig. 27. *E. procumbens.*

Fig. 28. *E. tuberosus.*

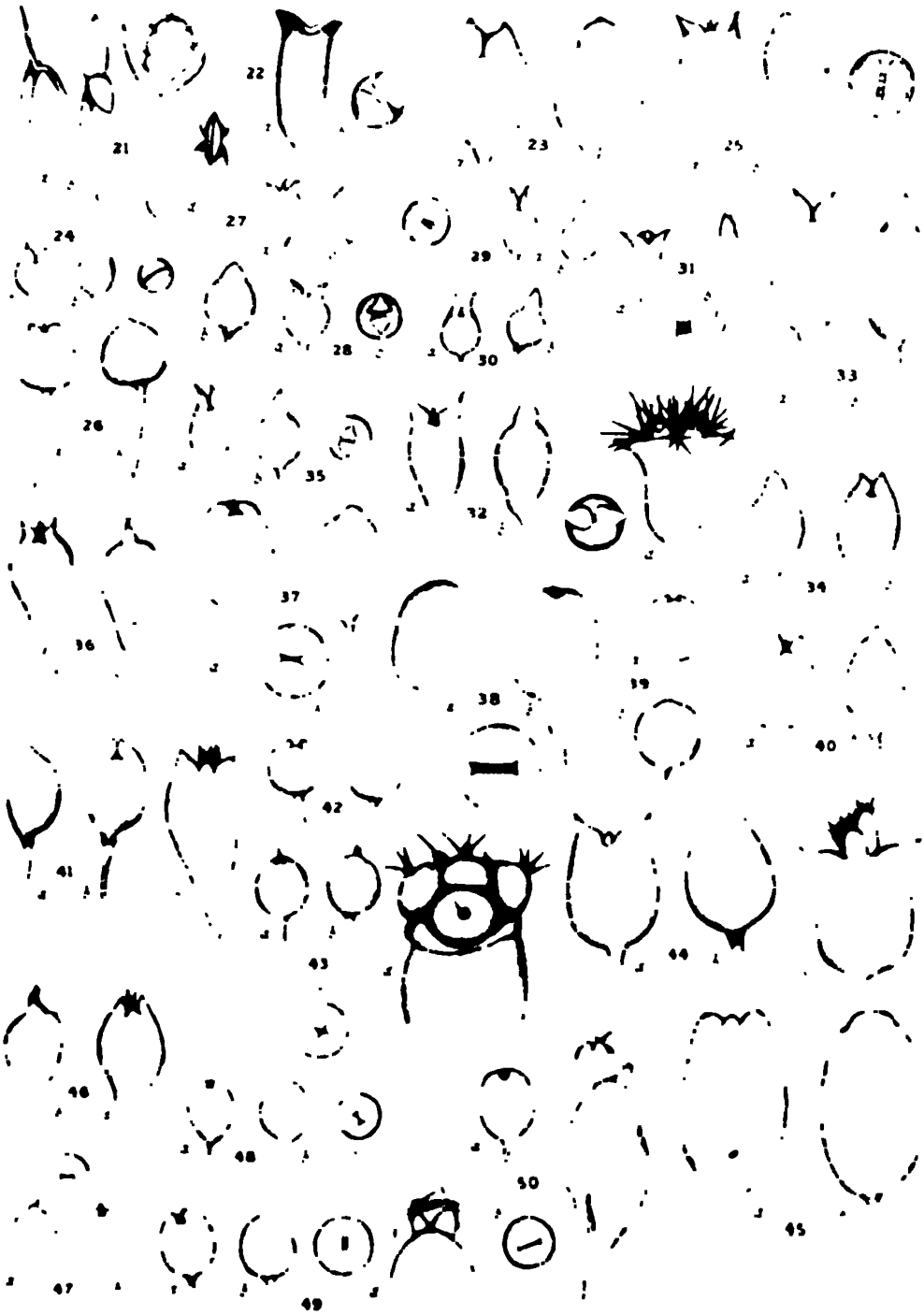
Fig. 29. *Echinopsis Zuccariniana.*

Fig. 30. *E. Eyriesii.*
Fig. 31. *Rhipsalis Cassytha.*
Fig. 32. *Echinocactus Wislizeni*: *d*, older specimen.
Fig. 33. *E. viridescens.*
Fig. 34. *E. texensis.*
Fig. 35. *E. longihamatus.*
Fig. 36. *E. cornigerus.*
Fig. 37. *E. ingens.*
Fig. 38. *E. horizontalonius*: *d*, older specimen to show epicotyl.
Fig. 39. *E. Williamsii.*
Fig. 40. *E. myriostigma.*
Fig. 41. *E. capricornis*: *c*, older specimen.
Fig. 42. *E. lophothele.*
Fig. 43. *E. Scheerii.*
Fig. 44. *Leuchtenbergia principis*: *c*, older specimen to show tubercles.
Fig. 45. *Mamillaria elephantidens.*
Fig. 46. *M. radiosa neo-mexicana.*
Fig. 47. *M. Sempervivi.*
Fig. 48. *M. Goodridgii.*
Fig. 49. *Anhalonium fissuratum*: *d*, older specimen to show tubercles.
Fig. 50. *A. prismaticum*: *c*, older specimen to show tubercles and smaller root.



W. P. Ganong, del.

GANONG. — CACTACEAE.



...versity Press... after...

Anatomy of the Seedling of Bowenia spectabilis, Hook. f.

BY

H. H. W. PEARSON, B.A.

'Frank Smart' Student at Gonville and Caius College, Cambridge, and Assistant Curator of the University Herbarium.

With Plates XXVII and XXVIII.

THE genus *Bowenia* was first described in the Botanical Magazine in 1863, from plants sent to Kew from Rockingham Bay, in that year[1]. The particular interest of the plant from a phylogenetic point of view is centred in its bipinnate leaf, a character which is not found in other Cycads. 'With the exception of *Stangeria paradoxa*, no more remarkable Cycadaceous plant has been discovered than the subject of our present plate, and like that plant it differs from every other member of its Order in the nature of its leaves, which present remarkable analogies with those of Ferns; whereas, however, the anomalous character of *Stangeria* is affected by the venation of the pinnules, which so exactly simulated those of a *Lomaria*, that two authors had (unknown to one another) referred it to that genus, the resemblance in the case of *Bowenia* is in some respects

[1] Curtis's Botanical Magazine, (3) Vol. xix, Tab. 5398. London, 1863.

[Annals of Botany, Vol. XII. No. XLVIII. December, 1898.]

carried further, inasmuch as the leaf is not simply pinnate as in *Stangeria* and other Cycadeae, but decompound, like a *Marattia*[1].' The similarity between the leaves of *Bowenia* and the coal-measure fronds of *Neuropteris* and *Alethopteris* has been considered by Stur as evidence in favour of the Cycadean affinities of the latter genera[2].

A point of great physiological interest is the development of numerous curiously-branched apogeotropic roots from the upper part of the main root. The general appearance of one of these roots is represented in Fig. 2, prepared from a photograph of an old root kindly supplied to me by the Curator of the Royal Gardens, Kew. A physiological examination of these roots was not undertaken: but an interesting feature is the presence of *Anabaena*-filaments in the majority of those examined. The Alga was found in all of a few roots obtained from Kew, and in some of those obtained from our own plants. As will be seen later, the method of occurrence of the Alga is very similar to that described by Reinke[3] in the subterranean roots of *Cycas*, *Ceratozamia*, *Dioon*, and *Encephalartos*.

No Alga was found in the subterranean roots of *Bowenia*; but, as the only material examined was obtained from the young plants, its general absence must not be concluded. It is worthy of notice that whilst in the cases described by Reinke[3] the Alga was found in portions of the tissue thickly covered by soil, and was thus placed very unfavourably for assimilation of CO_2, in *Bowenia*, on the other hand, it flourishes chiefly, if not entirely, in roots which are freely exposed to the atmosphere.

My thanks are due to Mr. Lynch for the supply of the seedlings used in this investigation, which were grown at the University Botanic Gardens. Figure 1 represents a seedling of seven months' growth. Except where specially mentioned, no plants older than this were examined. As

[1] J. D. Hooker, in Curtis's Botanical Magazine, loc. cit.
[2] A. C. Seward, The Wealden Flora, Part ii, p. 5. London, 1895.
[3] Reinke, 'Zwei parasitische Algen,' Bot. Zeitg., 1879, p. 473.

far as the supply of material would allow, comparison was made with younger seedlings: in the cases indicated the sections figured were taken from younger plants.

THE SEED. (Fig. 1, *s.*)

The embryo occupies an axile position in a copious starchy endosperm whose consistency is compact and almost brittle. The testa is horny and thin. In germination the cotyledons remain within the endosperm and function as an absorbent organ. The depletion of the endosperm is a process of long duration; in some of the oldest seedlings examined the endosperm was by no means all exhausted. At their distal ends the two cotyledons are united into a single club-shaped body which projects into the endosperm (Fig. 3, *f. cot.*). A transverse section through this region shows a well-marked line of separation between the two portions of the united cotyledons. The proximal portions and the petioles are free.

The cotyledons are bounded by a definite epithelial layer composed of cells which are elongated in a radial direction, and are rather smaller than the ordinary mesophyll-cells. These cells are filled with dense protoplasmic contents and have large nuclei. The mesophyll-tissue of the cotyledon is composed of large parenchymatous cells of a uniform size.

A single leaf-trace in the stem divides to form about four separate bundles which enter the cotyledons. One or more of these may divide again, so that from four to seven distinct bundles, arranged in an arc, are found in each cotyledon. The bundles are orientated normally, the phloem being on the dorsal side of the xylem. The larger quantity of the xylem is centripetal, a considerable number of centrifugal elements being also developed (Fig. 4). The centripetal xylem is usually somewhat extended in a tangential direction, beyond the general contour of the bundle: one or two elements on the flanks of this portion of the bundle are elongated tangentially and show characteristic reticulate markings on their

walls (Fig. 4, *tr.*). These outlying elements would seem to be of the same nature as those described by Worsdell in the cotyledon of *Cycas revoluta*[1]. The phloem has the usual characters, but contains no fibrous elements such as are so abundant in the root-phloem. The protophloem has not undergone any crushing; in other respects the cotyledonary bundle closely resembles that of the lower part of the petiole of the foliage-leaf. In all the preparations examined, the cotyledonary bundles were strictly collateral[2].

The plumule is very short and undergoes but a small development during the later history of the plant. The radicle gives rise to a strongly-developed tap-root (Fig. 1).

The Root.

The seedling possesses a well-developed root-system comprising a strong tap-root from which arise numerous lateral branches, some of which are apogeotropic.

The anatomical structure of the main root is in no way abnormal. The stele is usually four- or five-arch, and encloses a well-developed pith. Fig. 5 represents a transverse section of the stelar region; the section was taken from a point about 2 cm. from the apex of a root which was 5 cm. in length. It shows five distinct primary xylem-groups, and three well-marked groups of phloem. In older roots the masses of phloem and xylem are equal in number. The stele is bounded by an endodermis, distinctly marked in older roots, but indistinguishable in the section from which Fig. 5 was prepared.

The pericycle consists of four or five layers of cells. The wide cortex is composed of large parenchymatous cells of a uniform size, which, in the older roots, are densely filled with starch. Many of the cells of the cortex, particularly

[1] W. C. Worsdell, On 'transfusion-tissue.' Trans. Linn. Soc. Bot., (2) Vol. v, 1897, p. 307.

[2] A cursory examination of a preparation which Mr. W. C. Worsdell kindly showed me at Kew was insufficient to convince me of the presence of a concentric bundle in the cotyledon.

those of the outer cortex, and also several of the pericycle-cells, are filled with cluster-crystals of calcium oxalate. Stone-cells are rarely met with in the root.

Increase in thickness in the root is largely due to an irregular division of the cells of the ground-tissue. In the upper part of the root this primary thickening is especially obvious, particularly in the pith, and takes place to such an extent that the vascular bundles become considerably displaced.

A phellogen-layer appears in the outer cortex in the young root, before secondary thickening commences. This is quickly followed by a second layer of phellogen which arises in the pericycle immediately beneath the endodermis. All the tissue outside the inner periderm is quickly cast off. This corresponds with Reinke's observation on the formation of two periderm-layers in the root of *Cycas circinalis*[1]. A similar condition has also been observed by Gregg in the root of *Cycas revoluta*[2].

Secondary thickening occurs in a normal manner. Cambium first appears as an arc on the inner side of each group of phloem (Fig. 5). The ring of cambium enclosing the xylem-masses is soon completed, and simultaneously with this the second phellogen-layer commences to be formed in the pericycle.

As in other Cycads, the cambium-layer gives rise, not to homogeneous masses of xylem and phloem, but to radially-extended plates more or less widely separated from one another by medullary rays.

Secondary tracheids are produced more plentifully on the flanks of the masses of phloem, in the immediate neighbourhood of the primary xylem groups (Fig. 6, *xy.*[2]). A few tracheids are also formed inside the cambium-ring opposite the plates of secondary phloem. The secondary phloem is composed of radial plates, about four cells wide, each of

[1] Reinke, Morphologische Abhandlungen. Leipzig, 1873, p. 19.

[2] Gregg, Anomalous Thickening in the Root of *Cycas Seemanni*, Ann. Bot., Vol. i, 1887, p. 64, footnote.

which is made up of sieve-tubes, some parenchymatous tissue, and small groups of long, very thick-walled, fibrous elements (Fig. 7), which, as Worsdell[1] has pointed out in the case of the stem of *Macrozamia*, must have an important function in an organ so largely composed of parenchymatous tissue. A few cells filled with cluster-crystals of calcium oxalate are also found in the secondary phloem.

Opposite each primary xylem-group the cambium produces no secondary phloem, but a wide medullary ray (Fig. 6, *m. r.*). The medullary rays, as well as the pith and pericycle, afterwards increase rapidly in extent by primary cell-division, and consequently the arrangement of the secondary tissues, which is at first so regular as to be almost diagrammatic, becomes considerably disturbed.

No indications of growth in thickness of an anomalous character were observed.

The lateral subterranean roots arise in a normal manner from the main root, and are similar to it in structure, though the number of primary xylem-groups is commonly fewer than in the main root. Among the lateral roots a diarch or triarch structure is usually found.

The Apogeotropic Roots.

These roots spring from the upper portion of the main root, the first of them appearing a short distance below the origin of the cotyledons. Later ones are produced at points lower down, but apparently not in strictly acropetal succession. The term 'apogeotropic' is strictly correct as applied to these roots, for as soon as they emerge from the main root they grow vertically upwards. When full-grown, the apogeotropic root is from 2 to 4 cms. in length, and consists of a cylindrical stalk which bears above the surface of the ground a much-branched 'coralloid' head (Fig. 1, *a. g.*, and Fig. 2).

A remarkable feature of these roots is that their branching is of an exogenous type. At the apex is a merismatic

[1] Worsdell, The Anatomy of the Stem of *Macrozamia* compared with that of other genera of Cycadeae, Annals of Botany, Vol. x, 1896, p. 608.

tissue composed of small cells. The commencement of branching is indicated by a broadening of the apex, which is succeeded by the longitudinal division of the apical meristem into two apparently equal portions. But the dichotomy is only apparent, for one branch soon shows itself to be stronger than the other. None of the branches grow much in length before undergoing further branching of the same character, and to this is due the characteristic 'coralloid' appearance of the head of the root (Fig. 2).

The apogeotropic root arises, like the ordinary lateral root, endogenously opposite a protoxylem-group of the main root. A cylinder of cambium, continuous with the cambium of the main root, appears in the tissue of the young root before it has yet reached the surface, and gives rise to secondary xylem- and phloem-elements which are continuous with the corresponding elements of the main stele (Fig. 8, *a. ph.*^{2}, *a. xy.*^{2}). The primary structure of the stalk is similar to that of the ordinary lateral root. The stele is diarch, or more frequently triarch (Fig. 9). Primary growth in thickness is very much less than in the ordinary root: pith is usually absent, or, if present, very small in amount. A cambium appears on the inner face of each phloem-mass, but never—as far as was observed—forms a complete ring round the primary xylem-groups (Fig. 9, *cb.*). A single phellogen-layer arises in the external cortical tissue, an inner layer not being developed in the pericycle as in the ordinary root. Starch is found in the cells of the ground-tissue: many of the cells are filled with cluster-crystals of calcium oxalate, which are especially abundant in the cells of the pericycle and inner cortex.

The peripheral ('piliferous') layer of the coralloid head is composed of cells which are radially elongated, their outer ends being free from one another (Fig. 10, *p.*). This layer gives a villous appearance to the surface which is obvious to the naked eye. A phellogen-layer forms in the external cortex, beneath the papillose layer (Fig. 10, *pl.*), continuous with the phellogen-layer in the stalk. Eventually the papillose layer is cast off and the whole root is enveloped

in a continuous sheath of cork. As in the stalk, no phellogen is formed in the pericycle.

As has been stated above, most of the apogeotropic roots examined, particularly the older ones, contained colonies of *Anabaena*. The Alga is found in the intercellular spaces between the cells of a 'palisade-layer' which occurs midway between the periphery of the root and the stele (Fig. 11, *i. c.*). The 'blue-green' ring thus formed is visible to the naked eye in a transverse section. The Alga flourishes most abundantly in the tissues of the 'head'; but in the older roots it is present in a similarly well-defined ring for a considerable distance down the stalk, becoming less abundant lower down, and, in all cases yet examined, disappearing entirely above the junction of the stalk with the main root. The 'palisade-layer' is not seen in roots which contain no *Anabaena*, and it would seem that the characteristic appearance of the layer is due very largely to the lateral crushing, and consequent radial extension of its cells, by the pressure upon them of the developing colonies of *Anabaena* (Figs. 12 and 13). In roots in which the Alga is beginning to develop it occurs in small patches between the cells; as it becomes more abundant the cells are forced into the shape represented in Fig. 12, and finally the lumina become quite obliterated, so that neighbouring colonies are separated by radial partitions which can be recognized as collapsed cells only by treatment with re-agents. In this respect the method of occurrence of the Algal colonies in the apogeotropic root of *Bowenia* differs from that described by Reinke for *Cycas*, in which case a true palisade-layer of very definite form is present [1]. With the exception of a few isolated groups, the Alga is quite confined to this mid-cortical zone, which nowhere approaches nearer the surface of the root, and in many cases forms an uninterrupted layer parallel with the surface of the root.

The Alga is a typical *Anabaena*, showing characteristic heterocysts.

[1] Reinke, loc. cit., Bot. Ztg., 1879, see Figs. 2 and 3.

Transition from Root to Stem.

The normal structure of the root is retained to within a few mm. of the insertion of the cotyledons. The first indication of change is seen in the reduction of the pentarch structure to triarch: this is brought about by the disappearance of two protophloem-groups (see Fig. 5) and the subsequent union of the two adjacent pairs of protoxylem-groups to form two distinct masses. The result is, three groups of phloem, and three alternating groups of xylem, two of which represent a fused pair each. The diameter of the vascular cylinder meanwhile diminishes, as the constituent bundles of xylem and phloem pass in slightly towards the centre. The third and smallest group of protoxylem next passes inwards into the centre, and its elements become scattered in the pith between the two larger groups of xylem (Fig. 21); the two groups of phloem which were formerly separated by this third xylem-group (Fig. 21, *ph.*^{1}, *ph.*^{1}), pass towards one another, and eventually become united.

The structure of the cylinder at this stage is as follows:— It is of an elliptical form (Fig. 21), two well-defined protoxylem-groups being situated at the ends of the longer axis of the ellipse (Fig. 21, *p. xy.*), and the third group of protoxylem represented by a number of tracheids which are scattered in the pith between the polar groups of xylem (Fig. 21, *c. xy.*). On one flank of the xylem-plate is a single large group of phloem (Fig. 21, *ph.*) showing a distinct band of crushed protophloem (*p. ph.*). On the opposite side of the xylem-plate are two smaller groups of phloem (*ph.*^{1}, *ph.*^{1}) which were separated by the third group of protoxylem, and which will shortly unite to form a single bundle. Each of the three phloem-groups is bounded on its inner face by cambium, to which is due a considerable development of secondary phloem and a few secondary tracheids (Fig. 21, *xy.*^{2}). As far as could be ascertained, this structure is always to be found in the region of transition, though it is not always

preceded by a definitely triarch stage as described and figured in this case. When the triarch arrangement does not occur, the xylem-masses pass to the centre, as in the case of the triarch cylinder, and the phloem forms two masses, one on each side of the slightly-elongated band of xylem.

The concentration of the vascular mass in the centre of the hypocotyl proceeds, and the cambium becomes extended, and finally more or less completely surrounds a central mass of xylem. At this stage the centre of the stele is occupied by a mass composed of three groups of protoxylem (Fig. 14, *p. xy.*), no pith being present. The cambium gives rise to secondary xylem and phloem and wide medullary rays (Fig. 14): but the cambium and the tissues formed from it never completely surround the central mass of protoxylem. The diameter of the whole cylinder is, however, very small compared with that of the hypocotyl. The cylinder has this structure at the point of origin of the cotyledons, and for a very short distance (about ½ mm.) below it.

The Stem.

The plumule undergoes very slight development, and the stem is, in consequence, very short. The growing-point becomes displaced laterally by the leaves, and is enclosed in the sheathing base of the youngest of them.

At the insertion of the cotyledons the central vascular mass (Fig. 14) breaks up into two equal portions, from which are derived the cotyledonary leaf-traces. The remainder of each portion divides into several smaller bundles, which in the lower part of the stem are arranged in a more or less regular ring around the enveloped pith. Above this the stem-bundles pursue a very irregular and generally oblique course, meanwhile giving off traces which pass to the foliage-leaves.

The xylem is entirely centrifugal in origin (Fig. 15). As has been shown for other Cycads[1], the secondary wood is

[1] Worsdell, loc. cit., Ann. Bot., vol. x, 1896, &c.

divided up into narrow radial bands separated by medullary rays of equal width (Fig. 15, *xy.*²). The protophloem forms a wide band of crushed tissue (Fig. 15, *p. ph.*): the secondary phloem contains no fibrous elements such as are so abundant in the phloem of the root.

In the upper portion of the stem the orientation of the bundle is by no means constant, and occasionally bundles are found whose orientation is inverted.

The stem is surrounded by a layer of cork continuous with the pericyclic periderm of the root. The cells of the phellodern contain numerous cluster-crystals of calcium oxalate. The cells of the ground-tissue have undergone a considerable amount of primary division. Many large mucilage-canals traverse the stem in an obliquely longitudinal direction in the neighbourhood of the vascular bundles, though it is not easy to trace any constant relation between the direction of the canal and that of the bundle. The canals are not continued into the root: branches are given off which pass into the leaves, but they are not found in the cotyledons. A large mucilage-cavity is found near the stem-apex, with which the canals communicate.

The Leaf.

The leaf consists of a swollen leaf-base, a long petiole, and a bipinnate lamina (Fig. 1). The young leaf has well-marked circinate vernation: its younger portion is protected by brown uniseriate hairs, each of which consists of a short basal cell upon which is inserted a long terminal cell.

The leaf-base is crescent-shaped in transverse section (Fig. 16). The edges of the base of the youngest leaf fold round, and enclose the growing-point of the stem. A peripheral band of cork is formed in the ground-tissue (Fig. 16 *c.*). The vascular bundles are orientated normally, the xylem being on the ventral side. In structure the bundle of the leaf-base is similar to that of the leaf-trace in the stem (Fig. 15), except that a few elements of centripetal wood, which become more

abundant higher up the petiole (cf. Fig. 18), have appeared. The general ground-tissue consists of parenchymatous starch-containing cells, among which are scattered numerous mucilage-canals, particularly in the neighbourhood of the bundles (Fig. 16, *m. c.*); these are continuous with the canals of the stem, and higher up unite to form the single central canal of the petiole (Fig. 17, *m. c.*). Numerous cells containing cluster-crystals of calcium oxalate are present, especially in the phelloderm. In the upper part of the leaf-base an irregularly-arranged band of stereome appears in the peripheral cortical tissue, and afterwards attains a greater development in the petiole.

No periderm is formed in the petiole. The epidermis and two layers of cells immediately beneath it are composed of very thick-walled cuticularized cells, forming a peripheral band of stereome which is interrupted by stomata (Fig. 17). Beneath it is an irregularly-arranged band of stereome, continuous with the similar band in the leaf-base. The vascular bundles of the leaf-base have undergone some fusion, and the petiole contains, usually, four resulting bundles which are orientated towards the centre, around the single central mucilage-canal (Fig. 17). The vascular bundle contains some centripetal wood (Fig. 18, *cp. xy.*), which increases in quantity towards the upper end of the petiole (Fig. 19, *cp. xy.*). The protophloem is represented by a wide band of collapsed tissue (*p. ph.*). In the secondary petiole the bundle shows a still further increase of centripetal wood, and decrease in number of centrifugal elements. This preponderance of centripetal over centrifugal elements increases in the higher parts of the bundle until, in the lamina itself, the xylem is almost entirely centripetal, centrifugal elements being absent or one or two only (Fig. 20). Transfusion-tissue is possibly represented by one or two more or less transversely-elongated elements on the flanks of the centripetal xylem, which however do not show the markings characteristic of that tissue (Fig. 20, *tf.*). The inconspicuous character of these elements may of course be attributed to the age of the leaf: no old leaves were

examined with a view of determining the development of the transfusion-tissue. But it is equally probable that a poor development of this tissue is characteristic of *Bowenia*, as it has been shown to be in the case of *Stangeria*[1]. *Bowenia* and *Stangeria* are alike in that the venation of the leaf consists of closely-arranged sub-parallel members of a dichotomously-branched system, by means of which the leaf is well supplied with conducting-tissue: and under these circumstances it is reasonable to suppose that the specialization of transfusion-tissue is less necessary than in cases in which the leaf is not so well provided with ordinary conducting-tissue.

Engler's statement[2] that the anastomosis of the veins described by Schimper does not occur, is confirmed in the case of the leaves of the seedling-plant.

The general structure of the lamina of the adult leaf has been fairly fully described by Nestler[3]. With a single, not unimportant, exception, this account is correct for the leaves of the seedling-plant. He states (p. 356) that the stomata of *Bowenia* differ from those of all other Cycads in that they are found upon the upper as well as the lower side of the leaf. Though many leaves were examined, not a single stoma was found upon the upper side of the leaf; whilst stomata of the kind described by Nestler were very abundant on the lower side. In comparing these two accounts it should be borne in mind that our plants were grown under glass in the Tropical Pit, a condition which may have affected the development of the stomata.

It was perhaps to be anticipated that an examination of young plants of *Bowenia* would disclose the existence of primarily concentric vascular bundles, the presence of which would lend support to the phylogenetic evidence supplied by the bipinnate leaf. However, no indication of the existence of such bundles in any part of the plant has been found in the

[1] Worsdell, loc. cit., Trans. Linn. Soc. [2] Engler and Prantl, ii. 1, p. 9.
[3] 'Ein Beitrag zur Anatomie der Cycadeenfiedern,' Pringsheim's Jahrbücher, Vol. xxvii, 1895, p. 356.

course of this work, though Mr. Worsdell informs me that he has seen a concentric bundle in the cotyledon.

The apogeotropic roots seem to form the most interesting feature of the plant, whether from a morphological or physiological point of view. An account of the mode of life of the plant, and the kind of habitat in which it flourishes in its wild state, might throw some light upon the importance of the functions of these remarkable organs. There can be no doubt that the presence of colonies of *Anabaena* within the apogeotropic root is a normal circumstance for plants in cultivation, and there is no reason to suppose that the same does not occur in plants in the wild condition. The development of the Alga in the root is from above downwards, and it is not unreasonable to suppose that at some later stage than has been yet examined it extends into the subterranean roots, as Reinke[1] has described for other Cycads. That the union is of mutual advantage can hardly be doubted, as both the Alga and the tissue surrounding it are in an unmistakably flourishing state. The restriction of the Alga to a definite zone is a very remarkable fact, and the more so since the zone appears to be composed of quite normal cells of the cortex before the appearance of the Alga, by the activity of which its later characteristic form is induced. The entrance of the Alga must be effected by way of the papillose surface of the head of the root, though no indication of this was found. It is noteworthy, that in the older root, where the Alga is found in the most flourishing condition, it is as effectually excluded from the surrounding atmosphere by an uninterrupted layer of cork, as if the organ which encloses it were buried beneath the soil, as was the case in the roots described by Reinke.

In conclusion, I desire to express my thanks to Mr. Seward, at whose suggestion I commenced this investigation, and who has continually assisted me during its progress; to Mrs. Seward, who kindly executed the drawing from which

[1] Reinke, loc. cit., Bot. Zeitg., 1879.

Figure 1 was prepared; and to Dr. D. H. Scott, to whom I am indebted for valuable advice on several points that have arisen during the course of the work.

THE BOTANICAL LABORATORY, CAMBRIDGE.

EXPLANATION OF FIGURES IN PLATES XXVII AND XXVIII.

Illustrating Mr. Pearson's paper on *Bowenia*.

The following is a list of the abbreviations used: *a. g.*, apogeotropic root; *a. ph.*^{2}, secondary phloem of apog. root; *a. xy.*^{2}, secondary xylem of apog. root; *c.*, cork; *cb.*, cambium; *cf. xy.*, centrifugal xylem; *cp. xy.*, centripetal xylem; *cx*, cortex; *d. e.*, depleted endosperm cells; *e.*, endosperm; *end*, endodermis; *ep.*, epithelial layer of cotyledon: *f. cot.*, fused cotyledons; *fb.*, fibres; *g.*, stoma; *i. c.*, intercellular spaces, containing *Anabaena*; *m. c.*, mucilage canal; *m. r.*, primary medullary ray; *m. r.*^{2}, secondary medullary ray; *p. ph.*, protophloem; *p. xy.*, protoxylem; *p.*, papillose layer of apogeotropic root; *ph.*^{2}, secondary phloem; *pl.*, phellogen; *pc.*, pericycle; *s.*, seed; *s. c.*, stone cell; *s. t.*, sieve-tube; *st.*, petiole of cotyledon; *se.*, stereome; *t.*, testa; *v. b.*, vascular bundle; *xy.*^{2}, secondary xylem.

Fig. 1. Drawing of a young plant, seven months old, ½ natural size.

Fig. 2. From a photograph. An apogeotropic root. × 2.

Fig. 3. Diagram of a median longitudinal section through the seed at the stage shown in Fig. 1.

Fig. 4. Transverse section of the vascular bundle in the cotyledon. × 180.

Fig. 5. From a photograph. Transverse section of the stelar region of the main root of a young seedling. × 60.

Fig. 6. Diagram to illustrate mode of secondary thickening in the root.

Fig. 7. Portion of a transverse section of the root, to show the structure of the secondary phloem. × 140.

Fig. 8. Diagram to illustrate mode of origin of the apogeotropic root.

Fig. 9. Portion of transverse section of the vascular bundle of the stalk of the apogeotropic root. × 180.

Fig. 10. Portion of transverse section of the 'head' of the apogeotropic root showing the papillose layer. × 70.

Fig. 11. Portion of transverse section of the stalk of the apogeotropic root, to show position of the '*Anabaena*-layer.' × 68.

Fig. 12. Ditto. × 180.

Fig. 13. Portion of longitudinal section of the same. × 90.
Fig. 14. Diagram of a transverse section of the vascular cylinder of the hypocotyl.
Fig. 15. Transverse section of a leaf-trace bundle in the stem. × 180.
Fig. 16. Diagram of transverse section through the leaf-base.
Fig. 17. Diagram of transverse section through the petiole.
Fig. 18. Transverse section of vascular bundle in the lower part of the petiole. × 180.
Fig. 19. Transverse section of vascular bundle in upper part of petiole. × 180.
Fig. 20. Transverse section of vascular bundle of the leaf-lamina. × 280.
Fig. 21. Transverse section of vascular cylinder in the hypocotyl. × 68.

p.f

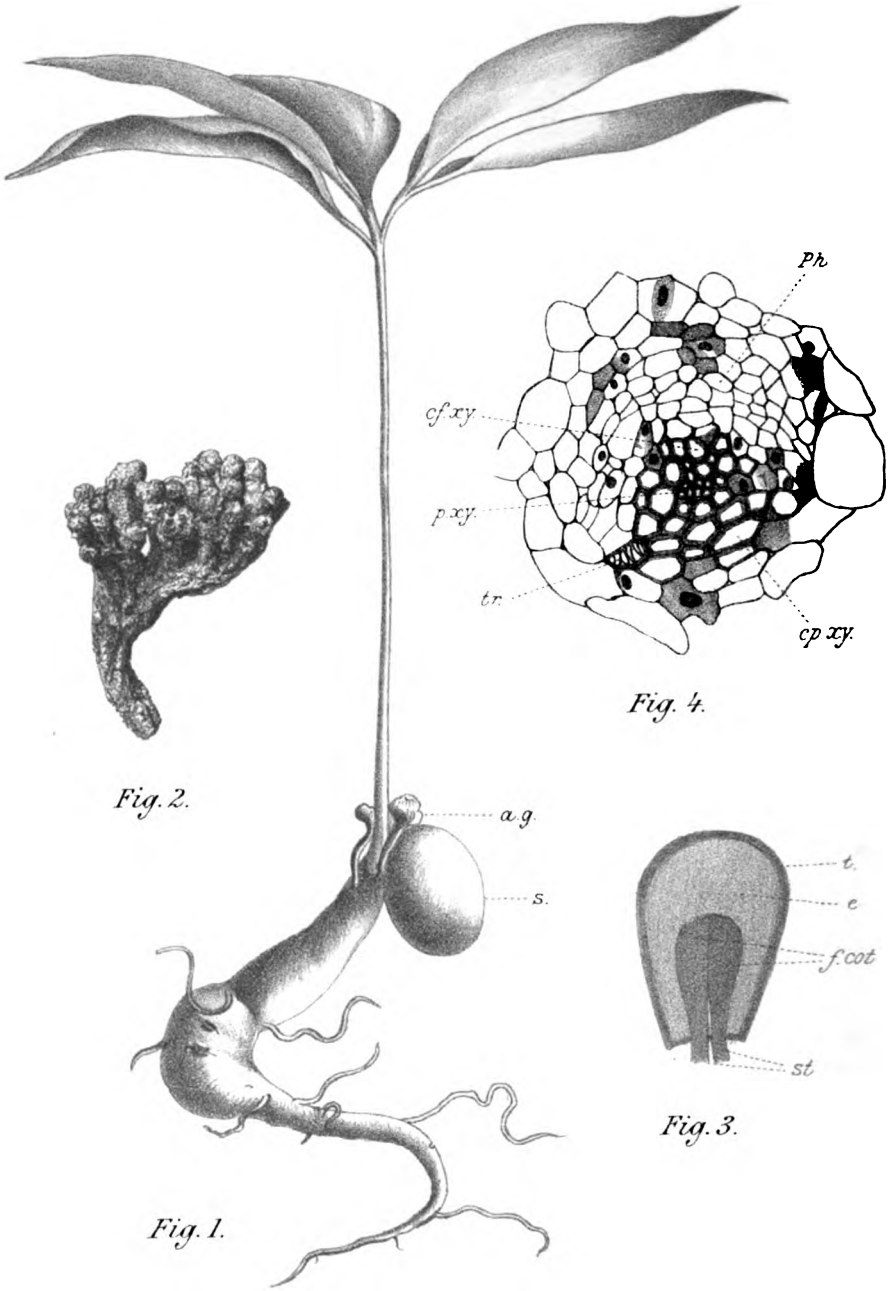


Fig. 4.

Fig. 2.

Fig. 3.

Fig. 1.

Figs 1, M. Seward, Figs 3,4,6-8 H.H W. Pearson del.

PEARSON. — BOWENIA.

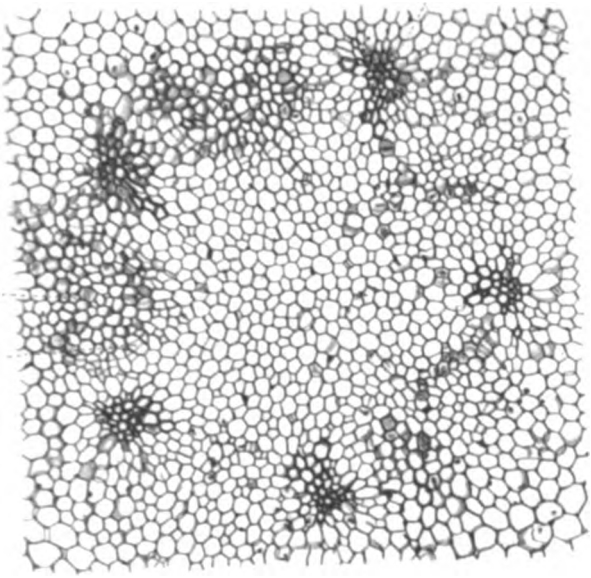


Fig. 5

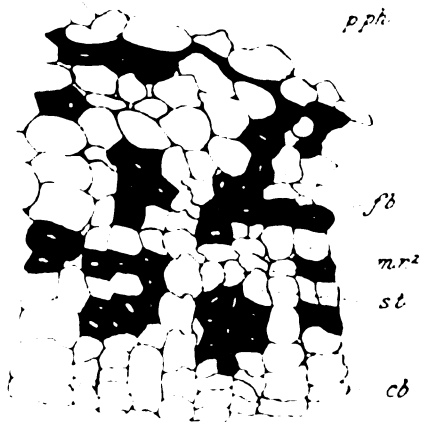


Fig. 7

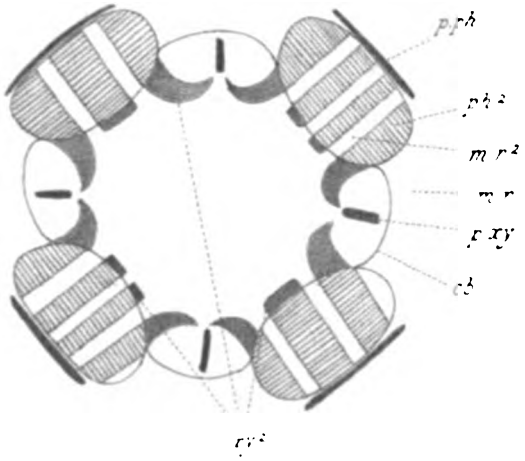


Fig. 6.

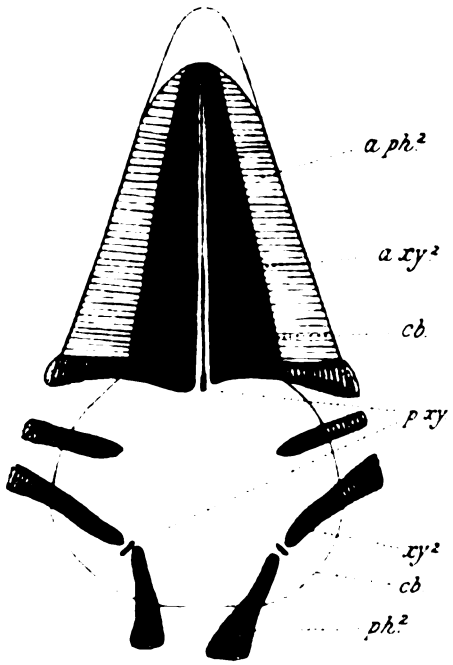
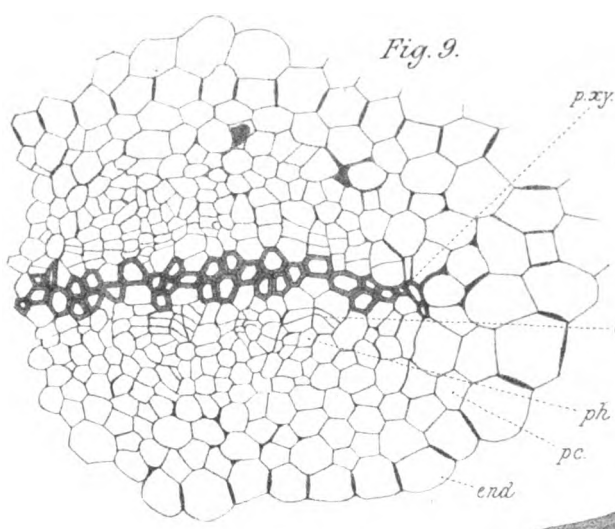
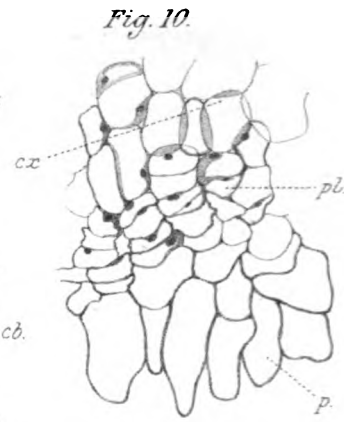
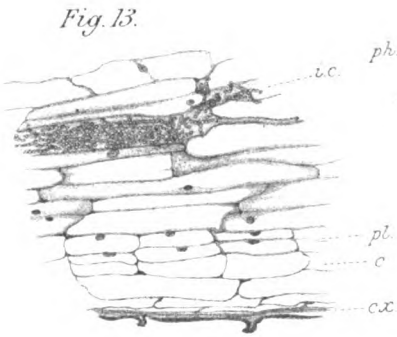


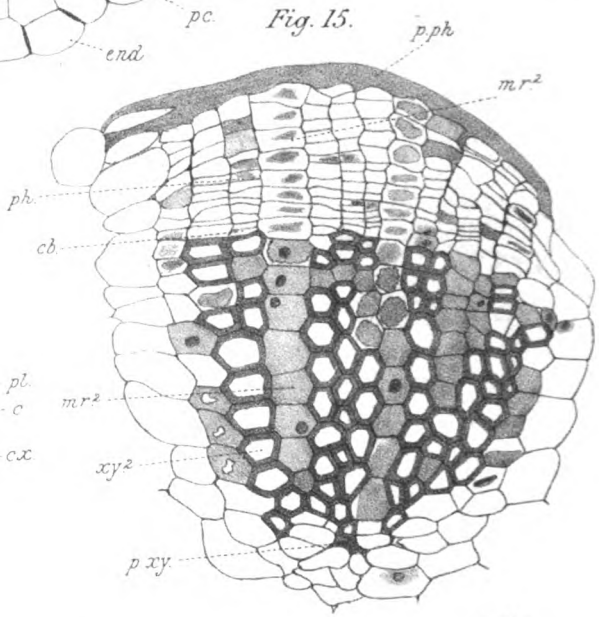
Fig. 8.

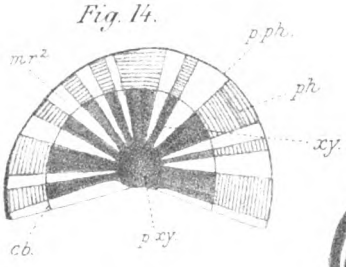
University Press Oxford

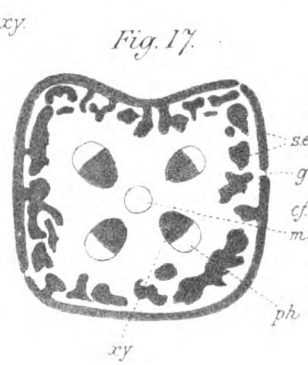


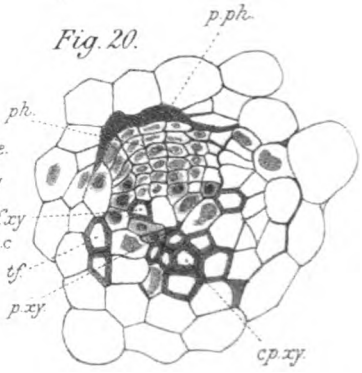


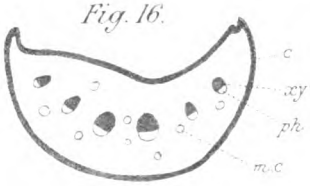












H.H.W.Pearson del.

PEARSON. — BOWENIA.

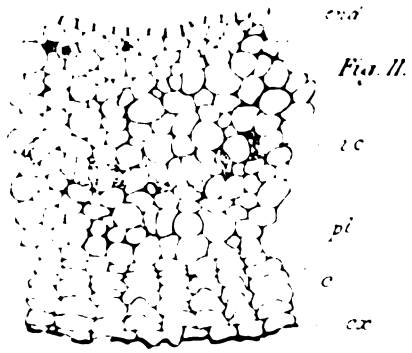


Fig. 18.

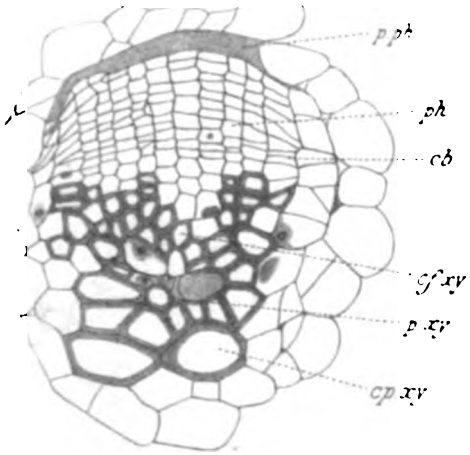


Fig. 19.

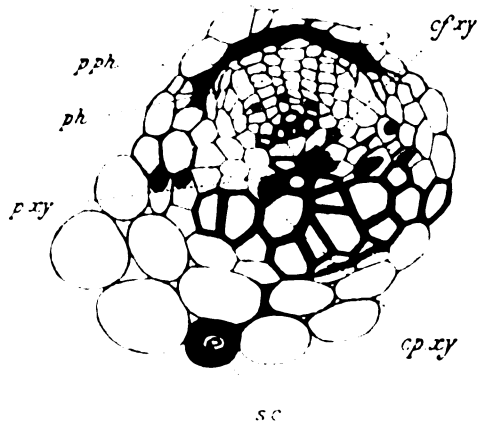
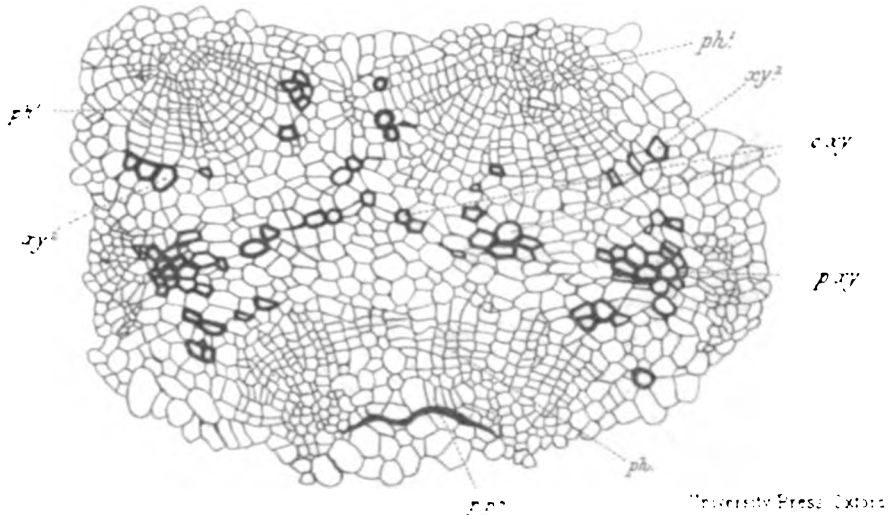


Fig. 21.



The Alcohol-producing Enzyme of Yeast.

BY

J. REYNOLDS GREEN, Sc.D., F.R.S.,

Professor of Botany to the Pharmaceutical Society of Great Britain.

IN a paper published in this Journal last year[1], I gave an account of some researches which I carried out with the object of confirming the discovery which had been announced some few months earlier by Dr. E. Buchner, that the alcoholic fermentation of sugar is effected by the activity of an enzyme or soluble ferment which, by appropriate means, can be extracted from the yeast-cell.

My experiments were made upon both high and low fermentation yeasts, procured without any special precaution from breweries actually at work, so that my inquiry might show whether in ordinary brewing operations the fermentation of the wort is due to an enzyme.

The yeasts I used were taken after the greatest activity of fermentation had subsided, and were kept in the laboratory for a day or two before using, so that they may be spoken of as being in the resting condition.

I followed closely the method of preparation described as his own by Dr. Buchner. Though I succeeded in pre-

[1] Annals of Botany, XI, No. XLIV, Dec. 1897, p. 555.

[Annals of Botany, Vol. XII. No. XLVIII. December, 1898.]

paring an extract which agreed in most particulars with his, my results were distinctly opposed to his as regards the existence of an enzyme. The liquid had the odour and the specific gravity of Buchner's, answered the same chemical tests, and deposited a heavy proteid coagulum in heating to 45°–50° C. It would not, however, set up even a small evolution of CO_2 when kept in contact with solution of cane-sugar at any temperature, nor would it manifest any other sign of the desired activity. It was capable, on the other hand, of inverting cane-sugar, and it digested its own proteids.

As a result of my inquiry made upon resting yeast under these conditions, I came to the following conclusion, which was given in my paper:—'For the present, therefore, I must contend, in opposition to Buchner, that at any rate our English yeasts do not contain any alcohol-producing enzyme.'

My researches during last year were made, as I have already said, upon yeast in the resting condition. During the present year I have carried out further experiments to supplement the former ones, and have worked with yeast taken for extraction at the moment of its greatest activity.

I obtained, through the kindness of Messrs. Combe & Co., a sample of a pure culture of *Saccharomyces Cerevisiae* from Hansen's laboratory. I cultivated this in beer-wort in an incubator, using about a kilogramme. The fermentation was conducted at a temperature of 20° C., and after a few hours it was very vigorous, the liquid frothing very energetically. While it was at its height, I removed the yeast from the wort, and rapidly strained it through a calico filter. The resulting pasty mass was then subjected to pressure in a screw-press till it was dry enough to crumble between the fingers. So prepared, the kilogramme of moist yeast originally employed weighed 100 grammes.

I had found in my former experiments that it was very difficult to grind the yeast-cells in the presence of sand, the latter being coarse enough to protect them from contact with the grinding surfaces: I therefore used no sand in the next stage of the process. Instead, I mixed the nearly dry

yeast with kieselguhr till the resulting powder was of the consistency of flour. This was then ground in an agate mill, procured recently from Lautenschläger of Berlin, which is used in the Pathological Laboratory here for grinding Bacilli. The mill is driven by water-power, and is capable of disintegrating the Bacillus of diphtheria.

This new apparatus materially shortened the operation of grinding, so that I was able to carry out the whole operation, from the collection of the yeast to the disintegration of the cells, in a few hours. I lay considerable stress upon this, for reasons which will appear later.

The absence of any enzyme in the resting yeast made it probable that, if present in the cells during active fermentation, it would not be very stable in the extract. In his publications Buchner has pointed out that this instability is one of the features of the enzyme as he prepared it. He speaks of it as being decomposed after exposure to the laboratory temperature for 24 hours, though he found it capable of greater resistance if kept at 0° C. He says, however, that he has kept it for a week without damage if it has been in contact with sugar. In order therefore to avoid every possibility of losing the enzyme, in case only a little should be present in the yeast under experiment, I took the precaution of extracting the ground mass, now a fine dry powder, with 100 cc. of a 10 per cent. solution of cane-sugar. During the progress of the grinding I examined every charge of the mill microscopically at intervals, and continued grinding until no intact yeast-cells were visible. It is of course unlikely that all were disintegrated, but certainly not more than 10 per cent. escaped intact.

On mixing the ground powder with the sugar-solution, and stirring it into a thin paste, I was struck by the fact that within five minutes an evolution of bubbles of gas took place in the paste, much after the manner in which yeast causes the fermentation in dough to occur. The bubbles rose slowly but constantly all over the surface of the paste. I rapidly mixed some unground yeast with kieselguhr, and

added some of the same solution of cane-sugar to compare the behaviour of the ground and unground yeast. In this control, a certain amount of gas appeared after a while, but it was much longer in being generated, and the amount was much less than in the other case.

I transferred the paste in a stone mortar to a refrigerator, in which I left it all night. The next morning the paste had become very porous, and had risen like so much dough, forming a dome-shaped mass nearly three times as large as the original volume of the paste. The control did not show at all an equal activity.

The paste was then wrapped up in a piece of fine sail-cloth and submitted to pressure. It was first squeezed in a screw-press, the weight being added very gradually till it reached 5–7 atmospheres per square inch. About 80 cc. of a yellowish liquid were thus extracted. It was then transferred to a hydraulic press, and the squeezing continued till a weight of about 500 atmospheres per square inch was applied to it. This great pressure only extracted about a further 15 cc.

My preparation so far agreed with Buchner's in the method of obtaining it, but the quantity of extract was very much less than his. I did not recover even all the extracting liquid, probably owing to loss by evaporation during the night.

The two extracts were kept separate during the further operations. Both were shaken up with a little kieselguhr and filtered through fine Swedish filter-paper. The resulting liquid gave the same reactions as Buchner has described for his own preparations.

Both before and during the filtration the extract was giving off small bubbles of gas, which formed a ring round the edge of the liquid in contact with the beaker.

I ascertained by microscopical examination that the filtrate was free from yeast-cells, and then I added to the 80 cc. of the first (lightly pressed) extract 50 cc. of a solution of cane-sugar of 40 % concentration. I put this mixture into a flask fitted with a mercury manometer; and to protect it from

disturbance by yeast-cells, if any should have escaped detection. I added an excess of chloroform, shaking it well. The flask had a side-tube, closed by a clip, by which I was able to equalize the pressure within and without in case any difference should appear. The outlets were closed with stoppers of sterilized cotton-wool, and the whole apparatus was sterilized carefully before putting the extract and sugar-solution into it.

After letting it stand about half an hour to recover from the shaking with the chloroform, the pressure was adjusted, and the flask left at the temperature of the laboratory. In less than another half-hour there was an internal pressure in the flask which displaced the mercury in the manometer ·5 inch. As time went on, this displacement increased until all the mercury was driven into the distal limb; and then, on gently shaking the flask, the generated gas escaped through the mercury-column.

At the end of twenty-four hours the chloroform had produced a copious precipitate of proteid matter in the flask. I rapidly extracted some of this precipitate, fearing it might be a growth of yeast. Microscopic examination showed that it was free from yeast-cells, and consisted of a fine amorphous powder. It is well known, of course, that yeast-cells will not grow in a saccharine liquid saturated with chloroform.

On the third day I divided the contents of the flask into two, and filtered half of it through filter-paper to get rid of the proteid precipitate. Fermentation was active in the liquid before opening the flask, the pressure supporting 1·5 inch of mercury in the manometer. I then put the filtered and the unfiltered halves side by side in similar flasks, and left them again at the temperature of the laboratory. Next morning the flask containing the filtered liquid showed very little alteration of level in the manometer, and on shaking only a slight amount of gas escaped from the liquid, only enough indeed to displace the level of the mercury about ·25 inch.

On shaking the flask containing the unfiltered liquid, on

the other hand, there was a copious escape of gas, and the mercury showed a permanent displacement of 1·5 inch.

It is interesting to note from this experiment that whatever gave rise to the evolution of gas was associated with the precipitate to a very large extent as soon as the latter was formed. The precipitate did not, however, remove from the solution all the gas-generating power, as the filtered liquid continued to give off gas, though in greatly reduced amount.

The evolution of gas in these two flasks was still going on at the end of a week from the beginning of the experiment. I then drew the gaseous contents of both flasks through baryta-water by means of an aspirator, and, as I expected, there was an immediate formation of barium carbonate, showing that the gas was CO_2.

I then distilled the contents of the flask, and found the liquid to have a specific gravity corresponding to the presence of about 1·5% of alcohol. The presence of the latter was confirmed by the iodoform reaction. The original extract, as prepared from the yeast, contained about ·3% of alcohol, so that during the fermentation rather more than 1% of spirit was formed.

The second extract, which was prepared by the heavier pressure as stated above, measured only about 15 cc. I filtered this through a porcelain filter under pressure and mixed it with sugar-solution, as in the other case: but instead of fixing a manometer to the flask I attached the latter to an arrangement by which the evolved gas was led through a tube containing calcium chloride to a set of potash-bulbs, so that its weight might be ascertained. Escape of water from the flask was guarded against by filling the connecting arm with sterilized cotton-wool. It gave off gas much more slowly than the liquid in the other flask, but there was still a noticeable activity. The flask and the bulbs were both weighed at the commencement of the observation, and again as the experiment proceeded. There was a continuous diminution in the weight of the flask, and a corresponding gain in that of the bulbs.

From these experiments I think there can be only one conclusion drawn. While the yeast-cells are active they secrete an enzyme, as Buchner says, which enzyme can be extracted by appropriate means. When so extracted it sets up fermentation in sugar-solutions under conditions which prevent the activity of living yeast. All the conditions of such fermentation were observed—the diminution of the sugar, the production of CO_2, and the coincident formation of alcohol.

The enzyme is easily decomposed; hence the necessity for rapid manipulation during the process of extraction. It possesses one of the characteristic properties of enzymes in general, in that it is largely thrown out of solution by the formation of an inert precipitate in the liquid which contains it.

The secretion of the enzyme by the cell is now shown to be intermittent, only taking place during actual fermentation by the yeast. It is soon decomposed when this activity ceases, so that resting yeast does not give it up to an extracting solvent. The completeness with which it can be extracted from the yeast-cell depends upon successful disintegration of the cell. I did not find that the enormous pressure employed by Buchner was necessary; indeed, the extract obtained by the comparatively low pressure of five atmospheres to the square inch, was more active than that obtained later by the use of the hydraulic press.

THE BOTANICAL LABORATORY, CAMBRIDGE.

The Nucleus of the Yeast-Plant.

BY

HAROLD WAGER.

With Plates XXIX and XXX.

LITERATURE.

THE following interesting observation was made by Nägeli in 1844 concerning the nuclei in the cells of Fungi: 'Structures resembling nuclei may be detected here and there in the cells of the Fungi. The fermentation-fungus in the must of Wine and in Yeast often exhibits a little nucleus of whitish mucus, lying on the membrane, regularly in each cell.'

This is the first reference made to the presence of a nucleus in the Yeast-cell. It may perhaps be doubted if Nägeli had before him the body known to recent observers as the nucleus; but it is interesting to find that under certain conditions the nucleus can be observed in the fresh condition of the cell, and is always in the position indicated by Nägeli.

Five years later the nucleus of the Yeast-plant was made the subject of investigation by Schleiden ('49), who applied reagents to determine its presence. In his work he states that, on treating Yeast with ether, alcohol, or potash, one

[Annals of Botany, Vol. XII. No. XLVIII. December, 1898.]

finds rounded delicate cells with a thin but clear cell-wall, containing clear contents with more or less delicate granules which singly or in groups occur on the inner surface of the cell-wall, and (almost?) always a large, round flat body (a cytoblast).

Brücke ('61) objected to Nägeli's observations, and says that, without wishing to maintain that Nägeli had not true nuclei before him, he is able, with improved apparatus and by observations on living material as well as on material treated with iodine and acetic acid, to state that no nucleus is visible, and remarks that nobody is justified in taking bodies of varying size and number, such as often occur, for nuclei.

Schmitz ('79), in his valuable paper on the nuclei of the Thallophytes, was able to show however, by the use of haematoxylin, that the Yeast-cell possesses a body which stains more deeply than the rest of the protoplasm. It occurs about the middle of the cell in the protoplasm near the vacuole, and he regards it as a nucleus.

Strasburger also ('84, '87) was able, by means of the same staining fluid, to demonstrate the nucleus described by Schmitz. He says that on fixing with picric acid and staining with haematoxylin, a rounded nucleus is to be seen near the centre, stained more deeply than the remainder of the cell-contents. In the living unstained condition a nucleus is not visible.

Zalewski ('85) found that the presence of a nucleus can be very easily rendered evident by placing the Yeast-cells in pure water for some time and staining with haematoxylin. The nucleus is oval in shape and possesses a small nucleolus in the centre. It stains more deeply than the other part of the protoplasm, which forms a dense layer round the nucleus. In budding cells the nucleus could not be discovered, nor in cells in which spore-formation was taking place; in these cases it was probably undergoing division, as a nucleus was observed in the fully formed spores and budded cells.

Objections to these observations were brought forward by Krasser ('85), who was able to observe granular structures

in Yeast-cells after staining, but could not recognize a nucleus, and was not able therefore to confirm the statements of previous observers. He points out that there is no specific staining reaction for the nucleus. The absence of a definite nucleus in the Yeast-cell is supported by the very rapid growth of the organism. The Yeast-cell possesses nuclein, but this is distributed through the protoplasm.

Both Hansen ('86) and Zacharias ('87) succeeded in demonstrating the presence of the body described as a nucleus by Schmitz.

Zimmermann ('87) states in his text-book (Morphology and Physiology of the Plant-cell) that he observed in a preparation stained with haematoxylin a deeply stained body which might be regarded as a nucleus, but he does not think this has been satisfactorily demonstrated. In a more recent communication, however ('93), he gives, in an account of the literature of the subject, this observation as supporting the view that the Yeast-cell contains a nucleus; and in a footnote referring to the doubt expressed by Krasser that Zimmermann had not been able to satisfy himself that a nucleus was present, he expressly states that in his figure given on p. 23 he regards the deeply stained body as a nucleus and the lighter body as a vacuole.

The evidence in favour of the presence of a nucleus is so far perhaps not very convincing or satisfactory, and Raum ('91), after an examination of a large number of various kinds of Yeast, came to the conclusion that it was not possible to state definitely that a nucleus is present in the Yeast-cell. On staining, with methylene-blue and Bismarck-brown, cover-glass preparations which had been allowed to dry and had been fixed by heat or corrosive sublimate, he found that the protoplasm stained brown and that a number of granules present in it stained black; whilst on staining with eosin and methylene-blue, the granules became dark violet. The number of granules varies in the different species and also their arrangement. They are not always present. Whether they are composed of nuclein is doubtful, but the author

finds that nuclein is present in Yeast-cells and was able to prepare and stain it. The granules do not appear to be concerned in any way with the process of budding or spore-formation.

In opposition to these observations of Raum and Krasser we have those of Moeller ('92), who shows that each Yeast-cell possesses one nucleus. This is a homogeneous body which possesses neither membrane nor nucleolus, and is capable of changing its shape. Its position in the cell varies; it may be central, or parietal, or at one of the poles of the cell. In the process of budding a portion of the nucleus makes its way from the parent cell through the opening into the bud. This then breaks off and rounds itself off as the nucleus of the newly formed cell. In order to observe the nucleus satisfactorily, the author makes use of cover-glass preparations fixed in a solution of iodine in potassium iodide. They are left in this solution for about twenty-four hours, then passed through water and dilute alcohol into absolute alcohol. Before staining they are soaked in saturated solution of picric acid for some time, washed in water, and stained in an alkaline solution of haematoxylin, or in one of the aniline dyes, fuchsin, methylene-blue, &c. The preparations are finally washed in water, dried, and mounted. From an examination of the spores, Moeller comes to the conclusion that they do not possess either a nucleus or a nuclear membrane, and that in consequence they are not true spores.

Mann ('92) observed in actively budding Yeast-cells, after staining in Ehrlich's acid haematoxylin, erythrosin, or eosin, a deeply stained granule which he supposes to be either a nucleolus or a nuclear chromosome.

Krasser however ('93) states again that the cells of Yeast contain nuclein, but that it is not contained in the body described as a nucleus by Moeller. It appears to be distributed in a finely divided form throughout the whole of the protoplasm. It is easily demonstrated macrochemically, but he finds considerable difficulty in demonstrating it by microchemical tests. He does not accept Moeller's view therefore

that the body described by him can be regarded as a nucleus, and in fact he failed to discover it in all Yeast-cells.

Moeller ('93) in a later communication points out that a better method of demonstrating the nucleus consists in boiling the cells in distilled water for one or two minutes, and then staining with haematoxylin by Heidenhain's method. In yet another communication to a different journal ('93 *a*) he gives an account of further observations which he has made to show that Krasser's contention that a true nucleus is not present is not the correct one, and reiterates his statement that the vegetative cells contain a distinct nucleus. He also finds that the spores contain a similar body. In the formation of the spore-nuclei, the mother-nucleus increases in size and becomes elongated and constricted. The ends separate from one another to opposite poles of the cell, the connecting thread breaks through and disappears, and two daughter-nuclei are thus formed. The division is a direct one. In contradiction to his former statement he finds that true spores are formed in the mother-cell: but whether the Saccharomycetes belong to the Ascomycetes, and to the group Exoasci, appears not yet proved; at present they must be regarded as *genera incertae sedis*.

Hieronymus ('93) introduced another difficulty into the solution of this vexed question by his discovery in Yeast-cells of a structure which he regards as similar to that which he has described for the Cyanophyceae. The protoplasm is full of angular granules which have a strong affinity for stains, and are so arranged that they form a thread interwoven in such a manner as to form a regular spiral or ball, which he calls the central thread. Sometimes this ball is located at one side of the cell, at others it expands and appears to pervade the whole of the cell; sometimes it is found separated into two portions, one at each pole of the cell, connected together by a single row of these granules. During the process of budding a portion of this thread passes into the daughter-cell.

Dr. A. Gortz, however, as quoted by Zimmermann ('93),

was unable to confirm the observations of Hieronymus, although he used the same methods and the same appliances, but was able to demonstrate the nucleus by the use of Merkel's solution and subsequent staining with fuchsin and methylene-blue. He did not complete his investigations, however, owing to the appearance of Janssens' paper.

Janssens ('93) states that not only do the cells of Yeast contain a nucleus, but that in the process of budding and spore-formation it divides karyokinetically, and the author was able to observe some stages of this, including the spindle-figure. He examined various Yeasts, including *S. Cerevisiae* and *S. Ludwigii*, to which he paid special attention. The nucleus possesses a nuclear membrane and a nucleolus. The latter is about one-third the diameter of the nucleus, and is homogeneous. In spore-formation the nuclear wall disappears from around the nucleolus. The first karyokinesis takes place longitudinally in *S. Ludwigii*, transversely in *S. Cerevisiae* I. The diaster and equatorial plate are easily seen. The second karyokinesis completes itself perpendicularly to the first, and the two spindles have a perpendicular direction to one another. The spores contain one nucleus, which becomes especially clear if the cells are placed during germination in water to which a little wort has been added.

Dangeard ('93 and '94), from a study of material which had been fixed by alcohol and stained with haematoxylin, also affirms that a distinct nucleus is present. The nucleus is placed in the layer of protoplasm surrounding the central vacuole. It is spherical in shape, possesses a thin membrane, and a large nucleolus which takes up the stain strongly. The process of division of the cell takes place by the formation of a bud on that side of the cell diametrically opposite to the nucleus. The nucleus passes to the point of attachment of the young bud and undergoes division which, according to the author, is in most cases direct. One-half then passes into the daughter-cell. No membrane is visible round the nucleus while this is taking place, but as soon as it comes into the daughter cell it develops the ordinary structure.

Eisenschitz ('95) raises a very interesting question: he finds that on cultivating Yeast for a day or two in beerwort coloured with methyl-green, congo-red, &c., the granules in the cell become stained. The granules are partly within and partly outside the vacuoles. The granules are of different chemical nature. The author thinks that the granules and vacuoles may be regarded as the preliminary stages of a nucleus.

Macallum ('95) is inclined to regard the existence of a nucleus in the Yeast-cell, in its usual condition, as extremely doubtful, and supports Krasser's view that nuclein is disseminated through the cytoplasm. On repeating Moeller's experiments, and using this observer's method, he found that now and then a structure such as is described by Moeller is present in *S. Cerevisiae*; but on comparing these preparations with others made by hardening and fixing in corrosive sublimate and staining with haematoxylin and eosin, he finds that this body is stained by the latter but not the former, and after fixation with Flemming's fluid appears to have no particular affinity for any dye. In *S. Ludwigii* there is in the great majority of cells a corpuscle which corresponds with the nucleus of Moeller, but which behaves towards stains in a similar manner to the above. A substance like chromatin in its reaction to staining fluids appears to be disseminated through the protoplasm. The distribution of assimilated iron-compounds in these cells confirms these results. In *S. Cerevisiae* the assimilated iron is, like the substance which absorbs haematoxylin, distributed through the protoplasm, and sometimes in the latter in the form of granules. In *S. Ludwigii* it is chiefly found at the periphery of each large vesicle when there are only a few of the latter; but when they are numerous, the cytoplasm gives a uniform reaction for iron corresponding in its depth to that given with haematoxylin. There appears to be present also a substance which constitutes corpuscles of a nucleolar nature, which stain with eosin, and give a marked reaction for iron, but do not stain with haematoxylin. The

final conclusion arrived at by the author is that 'there is no nucleus, although such an organ may occur in other stages' which he has not been able to observe.

Henneguy ('96) describes some observations made by him in 1886 on a red Yeast which had appeared accidentally among his cultures. This Yeast, which he examined in the fresh state and after being stained, exhibited very strongly a nucleus surrounded by a nuclear membrane and possessing a nucleolus.

Crato ('96) shows that in an elongated Wine-Yeast which he examined, physodes are present with the protoplasmic network. On staining with iodine, a compact body which stains yellow is seen to be present; this may be the nucleus.

Buscalioni ('96), in his observations on *Saccharomyces guttulatus*, describes the division of the nucleus. The resting nucleus is a homogeneous body which divides directly by constriction whilst budding takes place. The two daughter-nuclei remain connected together by a thin filament until one of them has passed into the bud. A similar method of division is followed in the formation of spores. The latter differs slightly from the former, and may be regarded as a much reduced form of karyokinesis; the former is a simple process of fragmentation.

My own observations ('97) showed that in *S. Cerevisiae* the nuclear body can be easily demonstrated by careful staining with haematoxylin, Hartog's double stain of nigrosin and carmine, or aniline-water solution of gentian-violet. It appears to consist, in the majority of cases, of a homogeneous substance, spherical in shape, placed between the cell-wall and the vacuole. The process of budding in a Yeast-cell is accompanied by the division of this nuclear body into two. The division is a direct one, and does not take place in the mother-cell, but in the neck joining it to the daughter-cell. When about to divide, the nucleus places itself just at the opening of this neck, and proceeds to make its way through it into the daughter-cell, until about half of it has

passed through, when it divides completely, and the two nuclei thus formed separate from each other towards the opposite sides of their respective cells. In the process of spore-formation the nucleus divides into four, each becoming the nucleus of a spore.

Janssens and Leblanc ('98) state that all cells of Yeast contain a nucleus which possesses a membrane, caryoplasm, and a nucleinated nucleolus. At the commencement of fermentation the nucleus is vacuolized, and presents the appearance of a vacuole containing a little sphere animated by Brownian movements. In the process of budding the nucleus divides indirectly in some cases, directly in others. In *S. Cerevisiae* the nucleolus is divided into two in the mother-cell in the neighbourhood of the bud. In the cells which are about to form spores one finds two nuclei. These use together, and the result is practically a fertilized egg-cell. This nucleus then divides by a reduced process of karyokinesis. This division is again repeated, and four nuclei are formed, each of which forms the nucleus of a spore.

Bouin ('98) states that the Yeast-cell contains in its normal condition a distinct nucleus. During fermentation this nucleus loses its clearness, and by putting out prolongations more or less clearly defined, it comes into close relation with the cytoplasm of the cell. Under the influence of an exaggerated concentration of the nutritive solution, or a reduction in the mineral elements, or by an increased temperature, the cells increase in size and become plurinucleate. This explains the observations of Hieronymus and others. The granules observed by these authors represent the nucleus which has become divided by a series of divisions not followed by cellular divisions. In the process of budding the nucleus divides more often directly than indirectly, but sometimes during budding, and always in the formation of spores, the division partakes of the indirect method.

In a recent paper, Macallum ('98) described a new method for the detection of combined phosphorus in tissues, and

pointed out that 'the method has resulted in demonstrating the presence of masked phosphorus in the chromatin of all animal and vegetable cells, in nucleoli . . . pyrenoids of Protophyta, &c. . . . It also shows that in non-nucleated organisms like the Cyanophyceae and *Saccharomyces*, the phosphorus-holding substance, or nucleo-proteid, although sometimes in the form of granules or spherules which have been taken for nuclei, is frequently dissolved in the cytoplasm.'

Errera ('98) states that he has been led to the following conclusions by a study of the cells of *Saccharomyces Cerevisiae*, part of which merely confirm former researches:—

1. A relatively large nuclear body exists in each adult cell.

2. Young cells contain no such body; a little later the old nuclear body divides, and one of its two daughters wanders through the narrow connecting-channel into the young cell.

3. After the division is complete, the two cells are still kept together by a mucilaginous neck-shaped pedicel, which appears not to have been noticed hitherto.

4. Carbohydrates are stored up in Yeast in the form of glycogen, which accumulates or disappears from the vacuoles very rapidly, according to conditions of nutrition and growth.

The evidence in favour of a nucleus in the Yeast-cell, as shown by these investigations, is very considerable, but it is very evident that its exact nature has not yet been determined. This is due, partly to its small size, partly, as we shall see later, to the difficulty of interpreting various structures which occur in the cell, and partly to the fact that the nuclear apparatus differs materially in structure from the nucleus of the higher plants.

METHODS.

Fixing and hardening.

Various methods of fixing and hardening have been tried, including the chrom-osmium-acetic mixture; chromic acid; solution of picric acid in absolute alcohol; picric and osmic

acid, and osmic acid alone; all of which give fairly good preparations: but the best results have been obtained by a saturated solution of corrosive sublimate, which should act for at least twelve hours, and by Gram's solution of iodine, which was used by Moeller, and subsequently by other observers, and which I have found to be of immense value in this work. The solution should remain on the Yeast for twenty-four hours.

The Yeast-cells may be fixed *en masse* in a small bottle, or cover-glass preparations may be made. Lindner ('97) observed that Yeast-cells behave in the same manner towards dyes as do Bacteria: like them they may be dried on a cover-glass and stained with various aniline-dyes. The spores also behave in a similar manner to the resting spores of Bacteria, and may be stained very easily with fuchsin.

It has been found by Janssens and Leblanc, and by myself, that completely drying up the living Yeast-cells on a cover-glass produces much contraction and disintegration of the contents. Janssens and Leblanc have found, nevertheless, that the liquid on the cover-glass may be almost completely evaporated without the Yeast-cells becoming quite dry, and that they stick sufficiently firmly to the cover-glass to allow the subsequent operations of hardening and staining to be carried out.

The method of fixing cover-glass preparations by heat, as practised by some observers, is not a good one, as has been already pointed out by others, but I should not say with Janssens and Leblanc that it is *absolument condamnable*. I have found it useful in certain cases, and have occasionally obtained very good preparations.

The method employed by me, however, is different from either of these. I first fix and harden the cells before making cover-glass preparations of them. I found that, even with the partial drying up, as practised by Janssens and Leblanc, the Yeast-cells showed signs of contraction of their contents; and further, that in the process of hardening and staining,

a large proportion of them were lost owing to their loose attachment to the cover-glass. The method I adopt obviates these difficulties, and in practice it is found that the cells may be completely dried up on the cover-glass without showing any signs of disintegration, if they have previously been well fixed and hardened. The method of procedure is a simple one. They are first of all placed in the fixing solution, either corrosive sublimate or preferably a solution of iodine in potassium iodide. They are then washed in water, 30% alcohol, 70% alcohol, and finally in methylated alcohol, which is constantly changed until all the iodine is washed out. Cover-glass preparations may then be made. A small quantity of the alcohol with Yeast-cells is placed on a cover-glass or slip. The alcohol is allowed to evaporate until the cells are nearly dry; then a drop of water is added and the Yeast-cells are thoroughly mixed up in it and spread out in a thin layer. When they have settled down the water is drained off, and they are then allowed to dry up completely. The cover or slip, with its layer of cells, is placed in water again for a few seconds and then stained.

Staining and Mounting.

Nearly all the methods of staining in vogue for nuclear work have been tried with more or less success.

Fuchsin and methyl-green. This is a very useful combination. It is prepared by adding an aqueous solution of methyl-green to an aqueous solution of acid fuchsin until a deep violet liquid is obtained. A drop of it placed on quite damp or wet blotting-paper should show a deep violet central spot surrounded by a narrow irregular blue or green ring. Cover-glass preparations stained in this for two minutes, then washed in water for ten seconds or so and mounted in dilute glycerin, show the nuclear body red, the cytoplasm blue-pink, and the vacuole and its contents blue, nearly the same colour as the protoplasm. The nuclear body may be perfectly easily made visible even in the most refractory specimens by this method, and especially in the

following manner, by which permanent preparations can be made. The cover-glass preparation is stained for two hours, washed in water, then in 70 % alcohol, then again in water and in 70 % alcohol, and so on until on examination under the microscope the protoplasm appears clear. It is then washed quickly in methylated alcohol and absolute alcohol, cleared in xylol and mounted in Canada-balsam. The nuclear body is coloured red and perfectly differentiated from the colourless protoplasm.

Methyl-green and eosin. By this combination the vacuole and its contents are stained green or blue, the protoplasm and nuclear body pink. The stain is allowed to act for one-half to two minutes; the preparation is then washed in water and examined in dilute glycerin. Permanent preparations may be made by drying up completely after washing in water, then clearing in xylol and mounting in balsam.

The successful application of these two methods depends to a large extent upon the judgment of the investigator in determining the right moment at which to stop the washing out in water or alcohol.

Haematoxylin. A dilute solution of Delafield's haematoxylin in water, allowed to act on Yeast-cells for several hours, which are then washed in water and 2 % alum solution, generally shows up the nuclear body quite clearly. The preparation may be washed in alcohol, cleared in xylol, and mounted in balsam. Good preparation can also be obtained by Heidenhain's iron-alum method. The cover-glass preparations are first of all mordanted in a 2·5 % solution of iron-alum in water for about three hours. They are then well washed in water and stained in a ·5 % solution of haematoxylin in distilled water for six to twelve hours, then they should be washed well in water, and soaked again in the iron-alum solution. In this the stained portions turn black, but are then gradually decolourized by a prolonged stay in the solution. After about two or three days, or sometimes more, the stain is found to have nearly disappeared from all parts of the cell except the nuclear body, but sometimes

a very prolonged stay in the solution is necessary in order to obtain a good differentiation. The preparations may be decolourized more quickly and effectively perhaps in alum solution.

Safranin. A solution in water to which a 3·5 % solution of aniline in water has been added may be used. The preparations remain in this for two or three hours; they are then washed in water, well washed in alcohol and acid alcohol, cleared in xylol and mounted in balsam. The nuclear body stains but slightly, the same as the protoplasm, but in good preparations the vacuole and its contents are coloured bright red.

Gentian-violet. A solution of this in aniline-water is very useful. Preparations should be stained for about half an hour; then washed in water and thoroughly washed out in 70 % alcohol, and finally mounted in balsam. In good preparations the nucleus stains pale reddish blue, the granules and vacuolar contents a deeper reddish colour. It is a very intense stain, and when carefully used gives good results. The combination of this stain with safranin and orange has not in my hands been productive of good results.

Fuchsin and methylene-blue. Stain first in carbol-fuchsin, then wash out in water and dilute alcohol, or very dilute solution of sulphuric acid, and subsequently stain in a dilute aqueous solution of methylene-blue. The nuclear body and spores are red, the protoplasm blue. This is not a very good combination for the study of Yeast-cells.

Carbol-fuchsin. Janssens and Leblanc give this as a good stain; but I have found that the combination of fuchsin and methyl-green, or fuchsin and methylene-blue, is far more effective. By itself the fuchsin stains somewhat diffusely. It is however an excellent stain for ripe spores when used hot, as in the staining of spores of Bacteria: preparations should be mounted in balsam.

Carmine and Nigrosin. This is used according to the method given by Hartog ('95) and gives fairly good results. It is necessary, however, to be very careful to wash out the

carmine very thoroughly as well as the nigrosin. A long stay in the alcoholic solution of acetic acid is generally necessary. This method is best adapted to the staining of cells which are to be cut by the microtome. I have also been able to obtain useful preparations showing the division of the nucleus in spore-formation by means of it.

Regina-violet. An aqueous solution is used. The cells are stained for about two minutes, then washed in water and 50 % of alcohol, and mounted in dilute glycerin. Nuclear body, vacuole, and protoplasm stain reddish, but the nuclear body and vacuole are differentiated from the cytoplasm by being rather more deeply stained.

Microtome-sections.

The fixed and hardened Yeast-cells may be stained, according to the method of Hartog, in carmine and nigrosin. The methylated spirit in which they are preserved is poured off and replaced by a very dilute solution of acetic acid nigrosin in 50 % spirit. After remaining in this a short time, the liquid is drawn off and replaced by Mayer's carmine; the liquid is well shaken and allowed to remain for several hours. The carmine is then poured off, and the Yeast-cells are washed several times in 30 % alcohol, which is then replaced by acetic nigrosin in very dilute solution. They should remain in this until sufficiently differentiated, which may be ascertained by occasionally placing a few cells under the microscope. When this has taken place the stain is drawn off, 30 % alcohol added, then 50 %, and they are gradually brought into absolute alcohol, in which they remain for an hour or so. The absolute alcohol is replaced by carbolized xylol, and the bottle or tube containing the stained cells is then placed on a water-bath, and pieces of hard paraffin wax added until all the xylol is evaporated and the cells are left in pure paraffin. They are then well shaken to ensure thorough penetration, and allowed to settle in a mass at the bottom of the tube or bottle. When this has taken place, the tube is plunged into cold water. The paraffin solidifies, and the

embedded Yeast-cells may now be got at by breaking the tube carefully. The paraffin-block thus obtained is trimmed, fastened to the microtome, and cut in the ordinary way. The ribbon is fastened to the slide by cement, the paraffin melted; the slide soaked in xylol, and finally mounted in balsam. In this way sections of the Yeast-cell are obtained in which the nuclear body can be very distinctly seen. Instead of carmine and nigrosin, haematoxylin may be used, according to Heidenhain's method.

Other Methods of Mounting.

Instead of being embedded in paraffin, the stained Yeast-cells may be mounted directly in balsam from the carbolized xylol. Care must be taken, however, to thoroughly break up the mass of cells with a brush on the slide in order to get an even regular layer. But perhaps the best method of doing this is to allow the cells, after carefully mixing them up with a brush on the slide, to dry up completely. By this means a single layer of cells only is obtained, if reasonable care be used. The slide can then be placed in xylol and mounted in balsam in the ordinary way.

Another method which is very convenient is to allow the xylol to evaporate from the bottle, leaving the mass of Yeast-cells perfectly dry. Small quantities of this dried stained Yeast can be handed out to students, or sent by post in small packets without coming to any harm. It may be very easily examined. A small quantity is crushed up in water and either examined at once, or the water may be drained off by means of blotting-paper and dilute glycerin added. If a permanent preparation is desired, the cells which have been spread out in water on the cover or slip are allowed to dry up completely; xylol is then added, and finally the preparation is mounted in balsam. This apparently rough treatment has very little, if any, effect upon the nuclear body; but for the investigation of the more delicate structure of the Yeast-cell it is not to be recommended.

Species examined.

The species of Yeast examined include—

1. *Saccharomyces Cerevisiae*—obtained from Leeds breweries.
2. Compressed Yeast—obtained from various agents in Leeds.
3. *S. Cerevisiae*—Hansen I.
4. *S. Ludwigii.*
5. *S. pastorianus.*
6. A red Yeast found in the air of the Laboratory and cultivated on gelatine.
7. *S. Mycoderma.*

I am indebted to the kindness of Professor E. Chr. Hansen for Nos. 3, 4, and 5, and I take this opportunity of tendering him my thanks for the specimens he was good enough to send me.

In order to make observations upon Yeast at different stages of fermentation, it was obtained fresh from a brewery; the wort was drained off, and cultures started in Pasteur's solution. In several series of investigations the Yeast was examined at the end of every hour, and specimens were fixed and hardened at the end of 1, 2, 3, 12, 16, 24, 38, 49, and 72 hours. Observations were also made upon Yeasts kept in sugar-solutions of various strength and in distilled water, all of which afforded useful information.

General Structure of the Yeast-Cell.

The contents of the Yeast-cell vary according to the conditions under which it is placed. In fresh actively growing Yeast the cell-contents are generally clear and homogeneous, with perhaps one or more bright refringent granules.

In young Yeast-cells and cells in an early state of fermentation—three or four hours in Pasteur's fluid—a vacuole or vacuoles can be seen. Each vacuole contains at least one refringent particle which is in a state of movement, and

in many cells there are two or more moving particles present. As fermentation proceeds these vacuoles disappear, and the protoplasm for a time appears homogeneous and clear; but as the culture-solution becomes exhausted the contents become more granular, large brightly refractive fat-globules appear in it, the protoplasm contracts away from the cell-wall, the cell-membrane loses its turgescent appearance, and the whole cell presents an appearance of disintegration.

Compressed Yeast-cells nearly always contain numerous brightly refractive granules. These are sometimes distributed regularly through the whole of the protoplasm; sometimes they are located only around the vacuole, or more or less densely grouped together on one side of the cell. These are the granules which Hieronymus regards as of the nature of a nucleus, and are called by him the central thread. They increase in number when the cells are placed in 5% sugar-solution, and sometimes almost completely fill the cell. The vacuoles may, as in other Yeast-cells, contain one or two brightly refractive granules which exhibit a Brownian movement (see Figs. 33–40).

The Nuclear Apparatus.

By the term nuclear apparatus is meant that portion of the Yeast-cell which appears to be set apart to perform the function of the nucleus.

According to Schmitz, Hansen, Strasburger, Moeller and others, the Yeast-plant possesses a nucleus of a simple structure consisting of a spherical homogeneous body placed on one side or near the centre of the cell. This body, which I propose for the present to call the nuclear body, can be very easily made visible by staining in methyl-green and fuchsin or in haematoxylin. To stain in methyl-green and fuchsin, a small quantity of fresh brewer's Yeast which has been fixed and hardened according to the method of Moeller by means of iodine-solution, is spread thinly over a cover-

glass or glass-slip and allowed to dry. A drop of the methyl-green and fuchsin mixture is then placed upon it and allowed to remain for two or three minutes. This is then washed off in water and the preparation examined in dilute glycerin under a one-sixth inch objective. The nuclear body will be seen coloured red and beautifully differentiated from the rest of the protoplasm, which remains colourless, or only slightly stained pink-blue. It is a perfectly homogeneous body even when observed under the highest powers of the microscope; but it is sometimes surrounded more or less completely by granules, which are stained blue or blue-pink, and these give it, especially when seen with inferior glasses or illumination, a granular appearance.

By means of haematoxylin it can perhaps be seen just as easily, but the preparation takes a longer time. I have found the following to be a good method. A cover-glass (or slip) preparation is taken prepared as above, and soaked for half an hour in a 2·5 % solution of alum. It is then well washed in water and stained for half an hour in a ·5 % aqueous solution of haematoxylin, and again well washed in water. It is now decolourized for half an hour or longer in the alum-solution and examined in dilute glycerin, or it may be passed through alcohols of various strengths and mounted in balsam. The nuclear body is by this method stained reddish blue or sometimes blue-red, and is beautifully differentiated from the protoplasm which remains very lightly stained. It is more clearly seen in dilute glycerin than in balsam.

Every cell of the Yeast-plant, except quite young buds, contains one of these nuclear bodies; very rarely are two to be found, except during budding or spore-formation. It is found in vigorously active Yeast, which has been fermenting for twelve hours, on one side of the cell, in close contact with the cell-wall; in a few cells it may be seen in a more central position, but very rarely exactly in the centre of the cell.

In cells which are stained very lightly, the nuclear body

appears to be surrounded on all sides by a more deeply stained membrane in close contact with it (Fig. 4). This seems in good preparations to be finely granular in nature, but it is not sufficiently definite to allow any positive statements to be made concerning it. It may perhaps be only a slightly denser portion of the nuclear body. The nuclear body is sometimes surrounded by granules which radiate into the surrounding protoplasm, giving it a star-shaped appearance which is described by Bouin ('98) as a nucleus.

On the whole the nuclear body appears to resemble the nucleolus of the higher plants more than anything else, and should probably be compared to it in function.

When stained as above described, or with the carmine-nigrosin combination, the nuclear bodies of different cells generally appear to be fairly uniformly stained and present a similar appearance in all; but in preparations stained with gentian-violet (see page 512), a difference in the affinity for the stain is observable in the nuclear bodies of various cells. In some cells the nuclear body is deeply stained; in others only faintly stained. It is generally clearly defined; but in badly stained or insufficiently washed-out preparations it may appear irregular in outline, as already described by Moeller and Bouin. This is due to the granular substance often found around the nucleus, sometimes in close contact with it, but which is not to be regarded as a part of the nuclear body, and in well-stained preparations is sharply defined from it.

The nuclear body can also be fairly easily rendered visible by allowing a dilute solution of iodine to run in gently under the cover-glass. The protoplasm stains first and the nuclear body is then visible as a pale unstained spherical body on one side of the vacuole. As the protoplasm becomes more deeply stained the nucleus becomes clearer.

If fresh Yeast be placed in Gram's solution of iodine for twenty-four hours, washed in water and placed in 30 % alcohol, and then in 70 % alcohol, the nuclear body can be very easily seen as being slightly more refractive than the rest of the protoplasm. It can also be very easily seen in specimens

preserved in methylated spirit; and most of the ordinary reagents used for fixing render it as a rule more or less visible [1].

In fresh Yeast it can in some cases be made out by careful examination under the one-twelfth inch objective, especially in compressed Yeast. As seen in the fresh condition, it is a pale slightly refringent spherical body. Its presence is often masked in compressed Yeast by the granules around it, but its position may be indicated by a slight flattening or indentation of the vacuole on that side on which it is placed.

The relation of Hieronymus' granules to the nuclear body is interesting. They may be easily observed if ordinary compressed Yeast be placed in very dilute sugar-solution and examined with a high power. Nearly all the cells will then be found to contain them. There may be only a few present, as in Fig. 33, or many, as in Figs. 34–40. In some cases they are grouped closely around the nucleus, as if connected in some way with it (Fig. 34), probably for purposes of nutrition. Sometimes the granules are found only on one side of it, sometimes on two sides or all round (Figs. 34 and 36), except in the region of the cell-wall and the vacuole. In addition to the granules around the nucleus we find a few or many in the protoplasm around the vacuole. In other cases the granules are not found grouped in this way round the nuclear body, but are distributed more or less regularly through the cell (Figs. 38, 39). In cells which had been kept in dilute sugar-solution for some hours, the granules were more commonly found grouped around the nucleus. After about twelve hours in a warm place in dilute sugar-solution, the granules increase in number, the protoplasm becomes vacuolar, and the nucleus takes up a position more in the centre of the cell, where it is surrounded by the granules on all sides (Figs. 39, 40). Sometimes the granules seem to

[1] Cells of *S. Cerevisiae* placed in a solution of alkanin for twenty-four hours or longer also show the nuclear body quite clearly, stained light red. The same body also gives, in cells hardened in alcohol, a definite reaction for phosphorus when treated according to the method described by Macallum ('98).

show the appearance of a coiled thread. This appearance is often observed just before the sporulation of a Yeast-cell.

On staining some of these cells on the slide with fuchsin, the granules can be seen stained fairly clearly, and in the midst of them the nuclear body faintly stained and rather difficult to make out. Some of Hieronymus' figures give one a very good idea of the appearance of these granules when stained, except that only in very few cases could any appearance of the nature of a coiled thread be seen. I have not yet been able to ascertain exactly what these granules are, but from the fact that some of them disappear on soaking in ether, and that they become coloured red in alkanin, they are probably of an oily nature; the others are probably proteid granules.

In addition to the nuclear body, there is present in the Yeast-cell in all species which I have examined, another structure which seems to be part of the nuclear apparatus. In young actively-growing cells this is represented by a vacuole containing a stainable substance, sometimes in the form of granules, sometimes in the form of a network, sometimes an irregularly shaped mass attached to the wall of the vacuole by fine threads (Fig. 1, &c.). In older cells it is represented by a more or less deeply stained granular network in which a small vacuole or vacuoles is sometimes visible (Figs. 22–27). This vacuole is taken for the nucleus by Janssens and Leblanc, who describe the nucleus of the Yeast-plant as consisting of a membrane, caryoplasm, and a nucleinated nucleolus. But according to them its structure is not always the same: in some cells the nucleus is a homogeneous body, but at the commencement of fermentation it presents in the fresh cell the aspect of a vacuole containing a spherule animated by Brownian movements. The moving spherule is regarded by the authors as the nucleolus, and is the same thing as the crystalloid of Hieronymus. In other words, the vacuole which can easily be seen in most Yeast-cells, without any special preparation, at the beginning of fermentation, is regarded by these observers as the nucleus.

The moving particle or nucleolus stains in the ordinary nuclear stains, and is supported to the wall by a caryoplasm of delicate threads.

These observations of the authors mentioned are in so far correct that, as previously stated, a vacuole with the structure they describe occurs in young cells; but whether it should be regarded as a nucleus or not is a question for further consideration. The presence of the nuclear body described by previous observers seems to have escaped their notice in the younger cells, although they have apparently seen it in older cells, for the body in these cells which they describe as the nucleolus is doubtless in many cases the *nuclear body* of previous observers. But as I have shown, by appropriate staining both a nuclear body as well as a vacuole can be seen in all cells which contain the latter, except in quite young buds; and when the nuclear body is seen through the vacuole we get an appearance which recalls at once the structure of the nucleus in higher plants (Fig. 7).

That there is reason for regarding the vacuole as possessing some of the attributes of a nucleus will be seen in what follows; for both Eisenchitz ('95) and Macallum ('95) had given indications of such a possibility in their memoirs; and I was able also to show ('97) that in addition to the nuclear body, there is a granular network present in the cell in close contact with it which resists the action of digestive fluids and is coloured intensely by nuclear stains.

In order to see the exact relation of the nuclear body to the vacuole it is necessary to examine Yeasts at different stages of fermentation, for there are two kinds of vacuoles, if we may speak of them as vacuoles—nuclear vacuoles, as I propose for the present to call them, and glycogen-vacuoles. The former are visible most clearly in Yeast-cells during the first few hours of fermentation; the latter are gradually formed as fermentation proceeds, and are generally of such a size as to completely fill the cell, leaving the nuclear body and a thin lining layer of protoplasm on the wall of the cell.

Yeast-cells taken three hours after the commencement

of fermentation, and stained according to the method already described, in methyl-green and eosin, for a few seconds, washed in water and examined in dilute glycerin, showed the following structure in different cases:—

1. A small vacuole (nuclear vacuole) containing granules and a delicate network stained green or blue, and a few granules which remain unstained; a layer of granular protoplasm stained pink, and a nuclear body in close contact with the vacuole, but *never* inside it, also stained pink or reddish blue (Figs. 1–4).

2. Small cells stained intense green all through, with a nuclear body (green), vacuole and granular contents (green), and homogeneous protoplasm (green) (Fig. 31).

3. Small cells with numerous vacuoles in a homogeneous protoplasm, and a nuclear body, all stained intense green, or in some cases with vacuoles and nuclear body green, protoplasm blue. Sometimes the nuclear body was found in the midst of the vacuoles, sometimes on one side of them (Figs. 28–30).

4. Cells with nuclear body blue, vacuole and contents blue, protoplasm pink.

5. Small cells with apparently no nuclear body, but with protoplasm and vacuole stained intense green. The absence of a nuclear body is only apparent however, for on carefully washing out the stain it is brought into view, and in methyl-green and fuchsin by sufficient washing out it is always visible, stained red.

6. Cells in which the vacuole is surrounded more or less completely by granules, which are stained blue. The vacuole contains very little stainable substance in most cases (Fig. 10).

7. Cells here and there with nuclear body blue, a vacuole present but not well marked, pink protoplasm, and a number of granules (blue) scattered through the protoplasm.

8. Cells in which a vacuole is not visible, but in its place a more or less regular granular network in contact with the nuclear body (Fig. 9).

Staining in methyl-green and fuchsin for a few seconds produces the same effect, but the nuclear body stains red, and the vacuole and its contents are not so clearly differentiated. Nevertheless, with careful staining good results are obtained. In successful preparations the nuclear body is bright red, the vacuole and its network deep blue, the protoplasm faintly stained blue. In some respects this is a more useful combination than methyl-green and eosin.

In aniline-water-safranin, the vacuolar network stains bright red, the nuclear body and the protoplasm light red.

In Delafield's haematoxylin—a solution which had been kept a long time—the nuclear body stains light red, the vacuolar network and granules deeper red. By the method of Heidenhain the nuclear body stains deep blue or black, the vacuole and contents lighter blue or black, the protoplasm remaining colourless or only faintly stained.

The nuclear body is always in close contact with the vacuole, and appears to be very intimately connected with it. Even when from whatever cause any contraction of the vacuole takes place, the nuclear body always remains in close contact with it, and one is never able to see any divisions between the two (Fig. 27). Granules inside the vacuole are often seen in contact with the nuclear body, and in some cases appear as if about to become absorbed into it. It seems likely that as the cells become older the contents of the vacuole may in part become absorbed into the nuclear body.

The appearance of the vacuole varies in different cells. In some cells it is large and contains very little stainable matter; in other cells it is small and often contains a dense mass of stainable substance. The stainable substance in the vacuoles is partly in the form of a network, partly in the form of granules. In some cells the network is distinctly granular, in others it consists of very fine, delicate threads. In some vacuoles there is sometimes a large, sometimes a small, central portion which stains deeply and is surrounded by delicate threads connecting it to the membrane of the

vacuole; this has the appearance of a nucleolus, but is not to be distinguished by its staining properties from the other substance in the vacuole, and its shape is also irregular in many cases, although it often is distinctly spherical. The network structure of many or most of the vacuoles recalls very distinctly the structure of the nucleus in the higher plants in the resting stage (Fig. 4), and its reaction towards stain is distinctly comparable to this also, although somewhat masked by the deeply stainable character of the protoplasm. The contents of the vacuole seem to contain a considerable amount of chromatin, as shown by its reaction towards stains, especially methyl-green, and its insolubility in digestive fluid. In some cells all the chromatin-substance appears to reside in the vacuole, in others it is diffused through the protoplasm, and in some cells it appears in the nuclear body. The first condition is found in young, actively-growing cells three or four hours after fermentation.

The nuclear vacuole may persist but a short time as such. At quite an early stage in the fermentation we find several cells in which a distinct vacuole is not to be seen, but only a granular network in contact with the nuclear body; and as fermentation proceeds still further, the vacuole disappears from nearly all the cells, leaving only this irregular granular network in contact with the nuclear body. On staining in methyl-green and eosin, both the nuclear body and the granular network around it are now found to stain intensely green or blue, apparently indicating that a portion of the green-staining substance has been taken up into the nuclear body (Figs. 21–27). The nuclear body at this stage is generally found closely pressed to the cell-wall by the mass of glycogen which has appeared in the cell as a result of an abundant supply of nutriment (Fig. 25).

With methyl-green and fuchsin, the nuclear body at this stage still stains red, but with a slight tinge of blue in most cases, and the granular substance in close proximity to it stains blue. The other contents of the cell stain pink. The granules are sometimes placed in a more or less regular

group on one side of the nuclear body, sometimes they surround it on all sides, and occasionally they are found distributed through the protoplasm (Figs. 21–27). In these older cells of Yeast, where the nucleus is restricted to the cell-wall by the large glycogen-vacuole, the nuclear body is sometimes surrounded by a vacuolar network in which granules may or may not occur (Fig. 26). This vacuolar network is sometimes very regularly placed around the nuclear body, which then looks as if surrounded by a halo, and has occasionally given rise to a false interpretation of its structure. This was especially well seen in some specimens of *S. Cerevisiae*, Hansen I, which had been sent to me in corrosive sublimate solution by Dr. Hansen (Fig. 26).

On treating fresh Yeast with digestive fluid (pepsin-glycerin) for twenty-four hours, a reduction in the stainable cell-contents is observed. In many cells a somewhat large, irregular granular mass is the only portion which stains deeply; in others, two or sometimes three such masses are observed, all connected together by deeply-stained granular strands. I was at first much puzzled by this, as no nuclear body was visible; but on repeating the experiment with a more careful staining, I found the nuclear body reduced in size and masked by the more deeply-stained granules around it. The granular substance in contact with the nuclear body varies much in size and shape, and is sometimes at some distance away from it, but is always connected with it by means of deeply-stained strands. The nuclear body stains much less deeply than this granular mass, and there seems to be no doubt that the latter consists of the much contracted and in part disintegrated nuclear vacuole. In older cells in which the nuclear apparatus had become restricted to the wall of the cell by the glycogen-vacuole, the whole mass—nuclear body and granules—stained the same green-blue colour in methyl-green and eosin and of the same intensity, an indication that at this stage in the development of the cell a considerable portion of the chromatin is taken up into the nuclear body.

In *S. Ludwigii* and *S. pastorianus* the structure of the

nuclear apparatus is in the main similar to that of *S. Cerevisiae* (Figs. 41–53). The nuclear vacuole seems to persist for a longer time however, as it does in compressed Yeast. The nuclear body is in close contact with the vacuole as a rule, but occasionally it may be separated some distance from it (Fig. 50). One often finds that a definite generally curved row of granules extends from the nuclear vacuole through the protoplasm to one or both ends of the cell (Figs. 43, 49, 50). In addition to the chromatin-vacuole, there are one or two large vacuoles present normally in a cell in the resting condition. As in *S. Cerevisiae*, there may be two or more small vacuoles present in young cells in place of one (Fig. 44, 48).

Origin of the Vacuole.

Young cells often contain numerous vacuoles surrounding the nuclear body (Figs. 28, 29, 30, 32). Some of these vacuoles are very small and are nearly filled up completely by a granule which stains an intense green, and thus appears to be of the nature of chromatin (Fig. 28). Under a low power these small vacuoles present the appearance of granules merely, and would be easily mistaken for the ordinary granules of a Yeast-cell; but under a high power their vacuolar nature can be easily made out in well-stained specimens. It is difficult to escape the conclusion that the vacuoles arise in some way in connexion with the granules. As the cell grows the vacuoles gradually fuse together to form the single vacuole in close contact with the nuclear body.

I have observed the same phenomenon in young Fungus-hyphae, probably *Mucor*, which constantly occur among the Yeast-cells in my cultures. In these hyphae the nuclei appear to be homogeneous in structure, and indeed stain in precisely the same manner as the nuclei of the Yeast-cells near them. In all the younger hyphae large vacuoles can be seen containing a substance staining green just as occurs in Yeast-cells, and these large vacuoles arise by the fusion of numerous smaller ones.

The single nuclear vacuole of the Yeast-cell thus has its origin in many cases from a fusion of numerous small vacuoles, and these smaller vacuoles are developed in all probability from the granules in the protoplasm.

There are some small cells however which contain only one vacuole. This, as we shall see later, probably arises by a division of the parent vacuole in the mother-cell.

Glycogen-Vacuoles.

Errera ('82, '85, '98), Clautriau ('95), and others have shown that the Yeast-cell contains glycogen. It is not equally abundant in all stages of growth. In the earlier stages of fermentation it is more abundant than in the later stages. As fermentation proceeds the glycogen is used up, and finally in old cultures practically no glycogen is to be seen, a few cells here and there only exhibiting the characteristic reaction when treated with iodine.

In the majority of cases the glycogen is located in a large vacuole in each cell which appears shortly after fermentation has commenced ; but during the first two or three hours the glycogen when present is mainly diffused through the protoplasm. In the glycogen-containing cells when stained with iodine, the nucleus is generally visible as a transparent, colourless or slightly greenish refractive body, sometimes spherical, sometimes flattened against the cell-wall (Figs. 23–25). Sometimes the entire cell-contents, with the exception of a thin lining-layer of protoplasm on the cell-wall, consist of this substance, which according to Errera ('85) doubtless plays the same part as starch in the higher plants.

So far as can be observed at present, the glycogen is located in a special glycogen-vacuole. It never appears, or only to a slight extent, in the nuclear vacuole. If a cell containing glycogen be stained for a few seconds in acetic methyl-green, the nuclear body and lining-layer of protoplasm become stained. If now a solution of iodine be added and the cells mounted in dilute glycerine, a beautiful double colouration

will be observed, differentiating the protoplasm and nuclear body and producing a clear definition of the glycogen-vacuole.

General Considerations.

We have thus seen that there are two structures in the Yeast-cell which together appear to represent the nucleus of the higher plants—the nuclear body and the nuclear vacuole. One of these—the nuclear body—is a permanent constituent of the cell; the other is not. The nuclear vacuole, when it is present, possesses some of the attributes of the chromatin-network of the nucleus of the higher plants, and in many cases presents a remarkable resemblance to it. The chromatin may be, and often is, very abundant. But the fact that the vacuole containing it may disappear, leaving the chromatin more or less completely disseminated through the protoplasm without the formation of chromosomes, except perhaps during the divisions leading to the formation of spores, seems to indicate that we are dealing with a body of much simpler construction than the nucleus of the higher plants; and it may be that this nuclear vacuole represents merely a store of chromatin-material for the use of the cell.

In the vegetative cells of other Fungi we appear to have at certain stages a similar structure. Vuillemin (Études biologiques sur les Champignons, p. 7) has shown that, in the hyphae of *Entomophthora glaeospora*, one finds here and there, in contact with a nucleus, clear spheres surrounded by a delicate membrane, which are not to be regarded as vacuoles but as being of a nuclear nature; and I have observed in the young hyphae of a Fungus, probably *Mucor*, a similar occurrence of chromatin-containing vacuoles. A careful examination of the vegetative cells of other members of this group of plants may possibly show that it is not uncommon.

The structure which we have called the nuclear body resembles in many ways in its reactions the nucleolus of the higher plants; and the fact that it may under certain con-

ditions contain chromatin-substance, recalls the structure observed in such cells as those of *Spirogyra* and perhaps the young cells at the apex of the root in *Phaseolus*, in which much if not all of the chromatin resides in the nucleolus.

We may I think therefore fairly conclude that the nuclear apparatus of the Yeast-plant consists of (1) a nucleolus, of homogeneous structure, the nucleus of the majority of previous observers; and (2) a store of chromatin which may occur either (*a*) in a network enclosed in a vacuole in close contact with the nucleolus, or (*b*) in a network in direct contact with the nucleolus, or (*c*) disseminated through the protoplasm. The chromatin is under certain conditions taken up into the nucleolus, viz. in spore-formation, or in the later stages of fermentation when it seems to be very abundant in the cell.

Further, if we regard it as a simple form of nucleus it may be either (1) a primitive structure representing an early stage in the phylogeny of the nucleus; or (2) a degenerate nucleus such as might be the result of the degradation of the Yeast-Fungi from higher forms, as is usually supposed to be the case; or (3) a special adaptation to the conditions under which the Yeast-plant lives and its rapid vegetative reproduction by budding.

The latter alternative is supported to some extent by the phenomena which occur during spore-formation in the Hymenomycetes. In the basidia, when the spores are formed, four nuclei are present, each possessing the normal structure of a nucleus. But before they pass into the spores everything seems to disappear except the nucleoli, which are left free in the protoplasm at the base of the sterigmata. This separation of the nucleolus from the rest of the nucleus is probably a special adaptation due to the necessity of passing through the narrow neck of the sterigma into the spore; and one can readily understand how valuable such an adaptation would be to the Yeast-cell with its very rapid vegetative reproduction, and how likely it is that such a condition of its nucleus should become a more or less permanent one as long as vegetative reproduction is unchecked.

As to the first alternative, the importance of the study of the cytology of the Yeast-Fungi and other low forms of Fungi as an aid in the elucidation of the very fascinating problem of the phylogeny of the nucleus need not be enlarged upon. There is in these lower forms a wide field of research open, which in the hands of a skilful chemist and cytologist may be very fruitful of results.

The comparison which might be made between the nuclear apparatus of the Yeast-plant and that of some of the Infusoria is an obvious one, but it seems to be only a superficial resemblance. The terms 'nucleus' and 'nucleolus' were rejected for the macro- and micro-nucleus of the Infusoria because they possessed neither the structure nor the physiological signification of the nucleolus and nucleus of ordinary cells (see Traité de Zool. Concrète, Delage and Herouard, 1896, p. 410, tome i). On the other hand, as we have seen, the nuclear vacuole and nucleolus of the Yeast-plant can be very definitely compared to the nucleus and nucleolus of ordinary cells.

The structure of the simple nuclear apparatus of the Yeast-cell may possibly afford some clue to the structure of the protoplast of the Bacteria and Cyanophyceae. The comparison, which may possibly be made of the central body of the latter with the nuclear body of Yeast, is at once apparent; and the appearances often presented by the larger Bacteria when compared with such elongated forms as *S. mycoderma*, *S. pastorianus*, *S. Ludwigii*, are very striking.

Budding.

In the process of bud-formation, both the vacuole, when it is present, and the nucleolus take part. In those cases where the nuclear vacuole is not present, the granular network, or group of granules which represents it, takes part in the process. The former is found chiefly during the earlier stages of fermentation, the latter during the later stages.

When the bud first appears on the mother-cell the nucleolus is found exactly on the opposite side of the cell with the

vacuole between it and the bud (Fig. 10). At first the young bud contains protoplasm only, but as development proceeds the nuclear vacuole begins to pass into it (Fig. 11). Then the nucleolus makes its way to the base of the opening of the mother-cell into the bud and at once begins to divide (Figs. 12–15). The vacuole at the same time divides. The products of division are unequal, the smaller portion is found in the daughter-cell, the larger portion in the mother-cell; but both portions remain connected together for some time by a granular thread (Figs. 14–16, 18). The division of the nucleolus takes place in a very simple fashion either in the mother-cell or, as is more commonly the case, in the neck connecting it to the daughter-cell (Figs. 16–21). In the former case it becomes elongated, and constricted in the middle, finally separating into two equal or nearly equal portions, one of which then makes its way through the narrow neck into the daughter-cell (Figs. 19–21, 52). In the latter case the nucleolus puts out a projection into the narrow opening between the two cells which makes its way into the daughter-cell. When about half of it has passed through, division takes place and two equal portions are formed, one in the mother-cell, the other in the daughter-cell (Figs. 16, 17, 53). This method is the one more commonly found in *S. Cerevisiae*; the other occurs more commonly in *S. Ludwigii* and *S. pastorianus*, and occasionally in *S. Cerevisiae* also. As soon as the two nuclear bodies are separated from one another they move away to opposite ends of their respective cells (Fig. 18); the granular thread between the two vacuoles is broken, and the division of the nuclear apparatus is complete.

When the nuclear vacuole is not present, the granular network in contact with the nucleolus undergoes a division into two more or less equal portions either in the mother-cell (Fig. 21) or in the neck joining it to the daughter-cell (Fig. 17); but in either case a granular thread is drawn out between them, and remains until all connexion between the two cells ceases.

The granules which thus pass into the young bud seem to develop in some way into the small vacuoles often found in young cells, by the fusion of which the single large vacuole is formed.

Spore-Formation.

The following is a very easy method of obtaining spores. Fresh compressed Yeast is placed in a dilute sugar-solution (about 5%) in an ordinary glass tumbler, and left to ferment at the ordinary summer temperature of a room. The surface of the liquid soon becomes covered with a scum, due to the fermentation at once set up, part of which is left sticking to the sides of the glass as the liquid evaporates. In the course of forty-eight hours the cells in the scum on the side of the glass begin to sporulate, and at the end of three days a large number of cells are obtained with spores in all stages of development. The method is a simple one, and has never failed to produce spores in large numbers. Spores are also easily obtained when a thin layer of compressed Yeast is spread over blotting-paper which has been soaked in 5% sugar-solution, and kept moist in a shallow dish covered by a glass plate. The sugar-solution seems to induce the formation of spores more freely than the plaster slabs soaked in water.

The spores can be easily demonstrated by staining cover-glass preparations in fuchsin as recommended for the spores of Bacteria. This stains only the ripe spores. The immature spores can be made visible by subsequent staining in a dilute aqueous solution of methylene-blue. In successful preparations the ripe spores are red, unripe spores blue, and the protoplasm light red.

The changes which take place in the cell, leading up to the formation of the spores, can be observed in fresh living cells, but it is necessary to stain them very carefully to observe the details of the process. The two best stains for the purpose are the combination carmine and nigrosin, and the solution of gentian-violet in aniline-water. Other stains have been found

useful, but these two have given me the most satisfactory results. It is not easy to get satisfactory preparations, however, owing to the difficulty of washing out the stain so that the cells are neither too deeply nor too lightly stained.

In the process of spore-formation as seen in the living cell, the protoplasm first of all becomes filled with bright refractive granules, most of them exhibiting a Brownian movement (Figs. 34–40). The nuclear body can generally be very easily seen, and one vacuole only is present in most cells. Then the vacuole disappears, its place being taken by two or more smaller ones, which are still further subdivided until finally the protoplasm appears structureless, or in favourable specimens exhibits the foam-structure described by Butschli. The nucleolus at the same time moves towards the centre of the cell and is surrounded on all sides by the bright granules. A condensation of the protoplasm towards the centre or one side of the cell now takes place, and gradually the spores are separated out by a division of this protoplasm into two or more rounded masses, each of which becomes surrounded by a membrane and gradually ripens into a spore.

From an examination of well-stained specimens, the changes which take place in the nuclear apparatus have been followed. Shortly after the Yeast-cells have been placed in the sugar-solution, the nuclear vacuole which contains a deeply-stained sphere or network of chromatin-like substance—the nucleolus of Janssens and Leblanc—begins to divide, first of all into two, then probably by further division into numerous smaller portions, until finally a delicate foam-structure of the protoplasm is produced and the chromatin-substance becomes distributed through the protoplasm. The nucleolus is found near the centre or one side of the cell at this stage and is slightly less deeply stained than the protoplasm (Fig. 54).

At this stage the nucleolus is very clearly seen, but in the earlier stages it is visible only after very careful staining. This has led Janssens and Leblanc to make, I think, a very curious but perhaps natural mistake. The primary division of the vacuole into two is described by them as the division

of the nucleus. At a later stage these two nuclei disappear, but in their place a single large nucleus is found. From this they come to the conclusion that the two nuclei fuse together again, and that the process may be regarded as a conjugation of two nuclei, transforming the cell into an egg, '*Ces deux noyaux, en se conjuguant, transforment cette cellule en un œuf.*' As I have shown above, their first two nuclei are produced by division of the vacuole, their single large nucleus found at a later stage is probably the nucleolus.

In the cell at this stage the protoplasm is uniformly stained rather more deeply than the nucleolus. In preparations stained with gentian-violet, the nuclear body is light blue, the protoplasm reddish blue, with a number of minute granules scattered through it.

This stage gradually passes into one in which the peripheral layer of the protoplasm loses its capacity for stains, leaving the central portion more deeply stained than before (Fig. 55). Numerous deeply-stained granules at the same time appear, chiefly around the outside of the deeply-stained central protoplasmic mass (Figs. 56, 57). This stage appears to correspond with that stage in the living condition of the cell which has been described as a condensation of the protoplasm either towards the centre or to one side of the cell. At the same time the nucleolus itself undergoes a change. Its central portion becomes more deeply stained than its peripheral portion (Fig. 56), presenting an appearance strikingly similar to that which I have observed constantly in the nucleoli of sections, stained with gentian-violet, of the root-apex of *Phaseolus*, just previous to the formation of the chromosomes. This stage is succeeded by one in which the central deeply-stained mass of protoplasm decreases in size, and at the same time the central deeply-stained granular mass in the nucleolus becomes larger (Figs. 57, 58). We are I think justified therefore in concluding that the increase in the stainable material of the nucleolus is due to the absorption of stainable substance from the surrounding protoplasm.

The nucleolus now begins to divide. Its outline becomes slightly irregular, and the deeply stained granular mass becomes more prominent. Then an elongation of the nucleolus takes place (Fig. 60), and we have gradually formed a long row of granules surrounded by a lightly stained blue substance, derived from the nuclear body, stretching across the cell either in a longitudinal or a transverse direction (Figs. 61–63). These granules gradually become separated into two groups by constriction, but they remain connected together for some time by a less deeply stained substance drawn out between them (Figs. 64, 65, &c.). Finally complete separation is effected and two daughter-nucleoli are produced (Fig. 68). Each one then divides again in the same manner, but in such a way that the line of division of one is perpendicular to that of the other, so that the two dividing nuclei often present the aspect of a cross (Figs. 69, 70, 72). Further divisions may take place leading to the formation of as many as eight nuclei (Fig. 71), but in most cells only four are produced (Figs. 73–75). Each of the four nucleoli thus formed becomes surrounded by protoplasm and a thin cell-membrane, and thus constitutes a spore lying free in the remainder of the protoplasm (Figs. 76–78). The spores are at first very small, but they gradually increase in size at the expense of the surrounding protoplasm, a thick cell-wall being produced around each, until finally they completely fill the mother-cell, the wall of which at this stage is in consequence not easily visible. They are then mature and enter upon a resting-stage.

The process of nuclear division just described may perhaps be regarded as a case of direct division in which the chromatin-substance is previously taken up into the nucleolus and separated out in the form of granules, which ultimately divide into two equal or nearly equal groups. But it may possibly be regarded as a very simple case of karyokinesis, if we look upon the granules as chromosomes, and the lightly stained substance which surrounds them during division as of the nature of a spindle-figure. The difficulty of observing all

the details of the division is, however, so great that one must be very cautious in attempting an explanation of the facts observed.

Summary.

It may be useful to give here a short summary of the conclusions at which we have arrived as a result of this investigation.

1. All cells of Yeast contain a nuclear apparatus.

2. In the earlier stages of fermentation this consists of a nucleolus in close contact with a vacuole which contains a granular chromatin-network, and exhibits a structure in many cases like the chromatin-network of the nuclei of higher plants.

3. In the later stages of fermentation the chromatin-containing vacuole may disappear, its place being taken by a granular network or a number of chromatin-granules, which may be disseminated through the protoplasm or grouped around the nucleolus.

4. The nucleolus is present in all cells. It appears to be a perfectly homogeneous body, which may, however, at times appear granular owing to the granules around it.

5. In young cells numerous chromatin-vacuoles are often found. These appear to fuse together to form the single vacuole which occurs in cells during the early and sometimes later stages of fermentation.

6. In the process of budding, the division of the nuclear apparatus does not exhibit any definite stages of karyokinesis. It must, I think, be regarded as a direct division of the nucleolus into two equal or nearly equal parts, accompanied by division of the chromatin-vacuole, network, or granules.

7. The nucleolus divides either in the neck joining the bud to the mother-cell, or more rarely in the mother-cell itself, one of the products of division passing subsequently into the bud.

8. In spore-formation, the chromatin disseminated through the protoplasm becomes absorbed more or less completely

into the nucleolus, which then divides by elongation and constriction into two. During the division deeply stained granules (chromosomes?) appear surrounded by a less deeply stained substance, which remains for a time connecting the two daughter-nucleoli together. This may perhaps indicate a simple intermediate stage of karyokinesis.

9. Subsequent divisions take place resulting in the formation of four (sometimes more) nucleoli. Each nucleolus becomes surrounded by protoplasm and a delicate membrane, and thus the spores are formed standing free in the remainder of the protoplasm.

10. The spores are at first very small, but they soon increase in size; the surrounding protoplasm becomes used up; the spore-membranes increase in thickness until at last in the mature condition they completely fill the mother-cell.

11. In *S. Ludwigii* and *S. pastorianus* the structure of the nuclear apparatus is similar to that in *S. Cerevisiae*, and its division during the process of budding appears to be also the same.

List of Papers Quoted.

NÄGELI, 1844: On the nuclei, formation and growth of Vegetable Cells. Translated from Schleiden u. Nägeli's Zeitschrift f. Wissenschaftliche Botanik, 1844, by Henfrey, Ray Society, 1845, p. 224.

SCHLEIDEN, 1849: Grundzüge der wiss. Botanik, 1849, p. 207. The principles of Scientific Botany; or, Botany as an Inductive Science; trans. by Lankester, 1849, p. 37.

BRÜCKE, 1861: Die Elementarorganismen: Sitzungsb. d. k. Akad. d. Wiss. zu Wien, Math.-Nat. Classe, Vol. xliv.

SCHMITZ, 1879: Untersuchungen über die Zellkerne der Thallophyten; Verhandlungen des naturhistorischen Vereines d. Preuss. Rheinl. u. Westfalens. Band xxxvi.

ERRERA, 1882: L'épiplasme des Ascomycètes et le Glycogène des Végétaux. Thèse, Brussels, 1882.

——— 1885: Sur l'existence du Glycogène dans la levure de Bière. Comptes-rendus, t. ci. p. 253, 1885.

KRASSER, 1885: Ueber das angebliche Vorkommen eines Zellkerns in den Hefezellen: Oesterreich. Bot. Zeitschrift, 1885.

ZALEWSKI, 1885: Ueber Sporenbildung in Hefezellen: Verhandlungen der Krakauer Akad. d. Wissenschaften, Math.-naturwiss. Section, Bd. xiii, 1885. See Bot. Centralbl. xxv, 1886.

HANSEN, 1886: Recherches sur la physiologie et la morphologie des ferments alcooliques (Meddelser fra Carlsberg Laboratoriet, Bd. ii, p. 152).

STRASBURGER, 1887: Botanisches Practicum, 1884 and 1887, p. 339.

ZACHARIAS, 1887: Beiträge zur Kenntniss des Zellkerns und der Sexualzellen. Bot. Zeitung, 1887 (p. 298).

ZIMMERMANN, 1887: Die Morphologie und Physiologie der Pflanzenzelle, Breslau, 1887, p. 25 (figure on p. 23).

LINDNER, 1887: Wochenschr. f. Brauerei, 1887, p. 773. See Jour. Roy. Mic. Soc., 1888, p. 156.

RAUM, 1891: Zur Morphologie und Physiologie der Sprosspilze; Zeitschr. für Hygiene, 1891, Vol. x, p. 1.

MOELLER, 1892: Ueber den Zellkern und die Sporen der Hefe, Centralbl. für Bakt. und Parasitenkunde, Vol. xii, 1892, p. 537.

MANN, 1892: The Embryo-Sac of Myosurus minimus, L.: A Cell-Study: Transactions and Proceedings of the Roy. Bot. Soc. of Edinburgh, 1892, p. 394.

MOELLER, 1893: Weitere Mittheilungen über den Zellkern und die Sprosse der Hefe, ibid., 1893, Vol. xiv, p. 358.

MOELLER, 1893 *a*: Neue Untersuchungen über den Zellkern und die Sporen der Hefen, Ber. d. Deutsch. Bot. Gesellsch., Bd. xi, 1893, p. 402.

KRASSER, 1893: Ueber den Zellkern der Hefe, Oesterreich. Bot. Zeitschr., 1893, p. 14.

HIERONYMUS, 1893: Ueber die Organisation der Hefezellen, Ber. d. Deutsch. Bot. Gesellsch., Bd. xi, 1893, p. 176.

JANSSENS, 1893: Beiträge zu der Frage über den Kern der Hefezelle, Centralbl für Bakt. und Parasitenkunde, Vol. xiii, 1893, p. 639.

ZIMMERMANN, 1893 : Sammel-Referate aus dem Gesammtgebiete der Zellenlehre. Beihefte z. Bot. Centralbl., Bd. iii, 1893, p. 420.

DANGEARD, 1893 : Sur la structure histologique des Levures et leur développement. Comptes-rendus Acad. Sci., 1893.

——— 1894 : La structure des Levures et leur développement : Le Botaniste, 1894, p. 282.

MACALLUM, 1895 : On the distribution of Assimilated Iron Compounds, other than Haemoglobin and Haematins, in Animal and Vegetable Cells : Q. Jour. Micro. Sci., 1895, p. 243.

EISENCHITZ, 1895 : Centralbl. f. Bakt. u. Parasitenk., 1895, p. 674 ; Jour. R. Mic. Soc., 1896, p. 218.

CRATO, 1896 : Beitrage zur Anatomie und Physiologie des Elementarorganismus : Cohn's Beitrage zur Biologie d. Pflanzen, Bd. vii, Heft 3, p. 495.

CLAUTRIAU, 1895 : Étude chimique du Glycogène chez les Champignons et les Levures : Extrait du tome liii des Mémoires couronnés et autres Mémoires publiés par l'Académie Royale de Belgique, 1895.

HARTOG, 1895 : On the Cytology of the Vegetative and Reproductive organs of Saprolegnieae : Trans. Roy. Irish Acad., xxx, 1895.

HENNEGUY, 1896 : Leçons sur la cellule : Morphologie et Reproduction ; Paris, 1896, p. 137.

BUSCALIONI, L., 1896 : Il Saccharomyces guttulatus Rob. : Malpighia, 1896.

WAGER, 1897 : The Nucleus of the Yeast-Plant. Report of the British Association, Toronto, 1897, p. 860.

JANSSENS AND LEBLANC, 1898 : Recherches Cytologiques sur la Cellule de Levure : La Cellule, t. xiv, 1898, p. 203.

BOUIN, 1898 : Contribution à l'étude du noyau des levures : Arch. d'anat. microscopique, t. i, 1898, p. 435.

MACALLUM, 1898 : On the Detection and Localization of Phosphorus in Tissue-Elements ; Proc. Roy. Soc., Vol. 63, 1898. See 'Nature,' Sept. 15, 1898, p. 483.

ERRERA, 1898 : Structure of the Yeast-Cell. A paper read at the Bristol meeting of the British Association in 1898 : also p. 567 of this number of the Annals.

WAGER, 1898 : Structure of the Yeast-Plant. A paper read at the Bristol meeting of the British Association in 1898.

EXPLANATION OF FIGURES IN PLATES XXIX AND XXX.

Illustrating Mr. Wager's Paper on the Nucleus of the Yeast-Plant.

All the figures have been drawn freehand, with the aid of Zeiss's apochromatic 2 mm. aperture 1·4, and oculars 8 (× 1000), 12 (× 1500), and 18 (× 2250). In most cases the outlines of the cells, &c., have been drawn with the aid of the camera lucida. Figs. 1–3, 5–21, 28–32, and 41–53 have been drawn from preparations stained in methyl-green and eosin; Fig. 4, safranin; Figs. 22–27, methyl-green and fuchsin; Figs. 33–40 from living cells; Figs. 54–57, 60, 62, and 63, gentian-violet; Figs. 58, 59, 61, 64, 65, 68, and 72–78, carmine and nigrosin; Figs. 66, 67, and 69–71, Heidenhain's haematoxylin.

Saccharomyces Cerevisiae.

Figs. 1–5, 7–12, 14–18, and 27–32, after three hours in Pasteur's solution.

Fig. 1. Cell showing nuclear body and vacuole with network and one deeply stained granule.

Fig. 2. Ditto showing three deeply stained granules in the vacuole.

Fig. 3. Ditto showing a vacuole not much larger than the nuclear body.

Fig. 4. Cell containing a vacuole which shows the nuclear-like network very clearly.

Fig 5. Cell with vacuole full of deeply stained substance, partly enclosing the nucleolus.

Fig. 6. Cell of compressed Yeast—the vacuole contains two deeply stained granules and delicate threads. Both nuclear body and vacuole are to some extent surrounded by unstained granules—Hieronymus' granules. Two hours in sugar-solution.

Fig. 7. Cell showing nucleolus as seen through a vacuole. The appearance is presented of a nucleus with nuclear membrane, &c.

Fig. 8. Cell showing nucleolus, in part surrounded by a dense mass of granules.

Fig. 9. Shows the nucleolus in contact with what appears to be a very definite chromatin-network. Whether this is contained in a vacuole or not, could not be made out clearly.

Fig. 10. Shows the position of the nucleolus at the time when the cell begins to bud. The vacuole in this case contains very little, if any, stainable substance, but is surrounded by deeply stained granules.

Fig. 11. The vacuole, with granular contents, is making its way into the young bud. The nucleolus still retains its position on the opposite side of the cell.

Fig. 12. The nucleolus has made its way to the opening between the bud and the parent-cell.

Fig. 13. Ditto. The vacuole in this case is surrounded by deeply stained granules. Seventy-two hours in Pasteur's solution.

Fig. 14. The nucleolus puts out a projection into the neck of the budding cell.

Fig. 15. Ditto, but a slightly later stage. The vacuolar contents are very abundant.

Fig. 16. Slightly later stage than Fig. 15, just previous to the complete division of the nucleolus. The vacuole is small and irregular in shape.

Fig. 17. The same stage as Fig. 16, but the vacuole has nearly disappeared, and in its place a deeply stained mass of granules nearly equally divided between the two cells.

Fig. 18. Complete separation of the newly formed nucleoli to opposite ends of their respective cells. A granular thread is shown drawn out between the two from the granular network.

Figs. 19–24. After seventy-two hours in Pasteur's solution.

Fig. 19. Shows the nucleolus beginning to divide by constriction in the parent-cell.

Fig. 20. Later stage than Fig. 19, the division is completed even before the vacuole begins to divide.

Fig. 21. Division of nucleolus in the parent-cell. A small vacuole only is present but there are a number of deeply stained granules, which are separated into two equal groups with a granular thread drawn out between them.

Fig. 22. Shows the glycogen-vacuole beginning to form. The chromatin-vacuole is still present with one deeply stained granule, and near the nucleolus numerous deeply stained granules are to be seen.

Fig. 23. Later stage—the large vacuole is the glycogen-vacuole.

Fig. 24. The nucleolus, chromatin-vacuole, and granules restricted to the wall of the cell by the glycogen-vacuole.

Fig. 25. *S. Cerevisiae*, Hansen I, shows nucleolus and granular network and large glycogen-vacuole.

Fig. 26. *S. Cerevisiae*, Hansen I, shows a nucleolus lying on the wall of the cell surrounded by a lightly stained vacuolar protoplasm, containing a few deeply stained granules. The remainder of the thin layer of protoplasm lining the cell is granular.

Fig. 27. Cell showing the gradual formation of the glycogen-vacuole, and the contraction of the chromatin-vacuole. Note the close attachment of the latter to the nucleolus. The protoplasm contains numerous deeply stained granules.

Fig. 28. Young cell with numerous small vacuoles, each enclosing a deeply stained granule, and some granules with a vacuole apparently just forming around each. The whole of the cell contents stain deeply.

Fig. 29. Young cell with three vacuoles, each containing a deeply stained granule. The cell contents stain deeply. The nucleolus is only visible after very careful staining.

Fig. 30. Ditto, with four vacuoles.

Fig. 31. Young cell with one vacuole, containing a deeply stained granule and delicate radiating threads. Near it a nucleolus only visible with difficulty.

Fig. 32. Young cell with nucleolus in the midst of a peripheral ring of granules. The whole cell is pervaded by a deeply stainable substance.

Figs. 33–40. Compressed Yeast examined in the living condition. The black granules represent the highly refractive granules described by Hieronymus.

Fig. 33. Half an hour after being placed in 5 % sugar-solution. The nucleolus is visible, and is indicated by a slight depression in the vacuole. Few refractive granules present.

Fig. 34. The nucleolus is surrounded by the refractive granules, which are now more numerous. Two moving granules in the vacuole. Two hours in sugar-solution.

Fig. 35. The bright granules more numerous. Two vacuoles present and a nucleolus. Two hours in sugar-solution.

Fig. 36. Shows three pairs of granules and small groups on each side of the nucleolus. Two hours in sugar-solution.

Fig. 37. Numerous vacuoles appear, as a preliminary to spore-formation. Three hours in sugar-solution.

Fig. 38. Later stage—the vacuoles are more numerous, the bright granules surround the vacuoles. Three hours in sugar-solution.

Fig. 39. The vacuoles disappear. The protoplasm, as shown by means of reagents, exhibits a foam-structure at this stage. Twenty-four hours in sugar-solution.

Fig. 40. Two groups of granules on opposite sides of the nucleolus in a hyaline protoplasm. Twenty-four hours in sugar-solution.

S. Ludwigii.

Fig. 41. Cell showing chromatin-containing vacuole and nucleolus.

Fig. 42. Ditto. Two lines of granules run from one end of the cell to the nuclear vacuole. The figure shows the nucleolus as seen above the vacuole, not inside it.

Fig. 43. Young bud just forming. Nucleolus beginning to divide. One row of granules stretching from one end of the cell to the nuclear vacuole.

Fig. 44. Cell showing two vacuoles and a nucleolus between them.

S. pastorianus.

Fig. 45. Cell showing two large normal vacuoles, and in the strand of protoplasm across the middle a nuclear vacuole and a nucleolus on one side.

Fig. 46. Shows the nucleolus as seen from above. On focussing down the vacuole could be seen.

Fig. 47. Cell showing nucleolus as seen through the vacuole.

Fig. 48. Cell with nucleolus and three small chromatin-vacuoles.

Fig. 49. Cell showing nucleolus at one end surrounded by granules which are connected to a small vacuole at the other end by a deeply stained row of granules.

Fig. 50. Nucleolus in the middle of the cell in contact with a curving line of granules running from a chromatin-vacuole at one end to the other end of the cell.

Fig. 51. Cell with young bud. The nucleolus is in contact with a vacuole containing a deeply stained granule and network.

Fig. 52. Later stage than Fig. 51; the nucleolus is beginning to divide.

Fig. 53. Case of budding in which the nucleolus is about to divide in the neck joining the two cells. The daughter-cell contains a chromatin-vacuole in close contact with its share of the nucleolus.

Spore-formation.

Fig. 54. Cell just at commencement of spore-formation—protoplasm reddish blue, nucleolus light blue.

Fig. 55. Cell in which the deeply stainable substance is becoming concentrated in a central mass of protoplasm. The nucleolus stains light blue as before, but a little more deeply.

Fig. 56. Later stage. The nucleolus shows a deeply stained granular substance inside or in close contact with it.

Fig. 57. The deeply stained granular mass inside or in contact with the nucleolus has increased in size. The deeply stained central portion of the protoplasm is surrounded by granules.

Fig. 58. Later stage. The deeply stained mass of protoplasm is smaller.

Fig. 59. Still later stage. The deeply stained nucleolus is now the most prominent structure.

Fig. 60. Commencement of division. The nucleolus and its deeply stained mass begin to elongate.

Fig. 61. The deeply stained granular mass at a later stage in the process of elongation.

Fig. 62. The row of deeply stained granules stretching across the cell, surrounded by a faintly stained substance.

Fig. 63. Shows the gradual accumulation of the granular substance at both ends to form the daughter-nucleoli.

Fig. 64. Complete separation has now taken place.

Fig. 65. Later stage in the division; the two daughter-nucleoli are still connected together by a less deeply stained substance.

Fig. 66. Division-stage, as shown in a preparation stained with haematoxylin, Heidenhain's method. One portion of the protoplasm is still shown more deeply stained than the other, with a distinct line of separation between them.

Fig. 67. Same method of preparation. The division is transverse.

Fig. 68. The two groups of deeply stained granules completely separated, each surrounded by a less deeply stained substance with a faintly stained granular thread drawn out between them.

Fig. 69. Division into four.

Fig. 70. Slightly later stage than Fig. 68.

Fig. 71. Shows a cell in which division is taking place into eight nucleoli instead of four.

Fig. 72. Four groups of deeply stained granules, still connected together by less deeply stained substance. The division apparently has taken place at one end of the cell.

Fig. 73. Stage similar to Fig. 71, but the groups of granules are more irregularly distributed at one end of the cell.

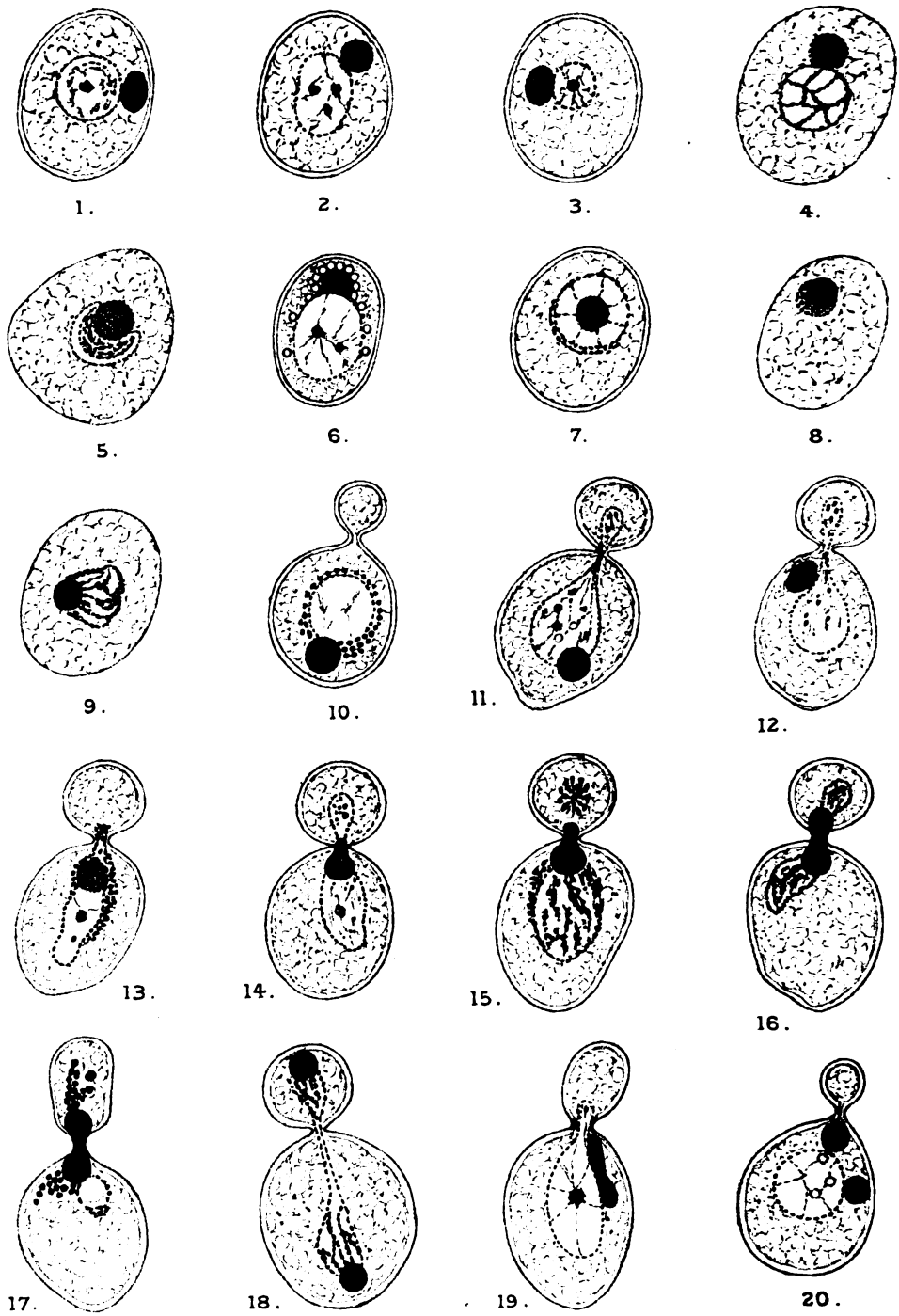
Fig. 74. The four products of the previous two divisions are now lying in a slightly more deeply stained portion of the protoplasm at one end of the cell.

Fig. 75. Later stage than Fig. 74. The spores beginning to separate out.

Fig. 76. The spores now visible, each with a distinct outline due to the presence of a thin membrane.

Fig. 77. Later stage. The membrane is more distinct.

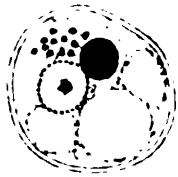
Fig. 78. Same stage as Fig. 77, but five spores shown, two smaller than the others. From observations made recently I am inclined to think that these two fuse together to form one.



H. Wager del.

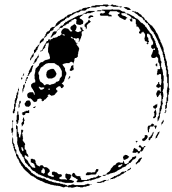
WAGER. — YEAST NUCLEUS.

21.



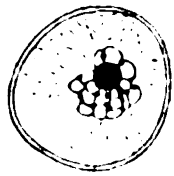
22.

23.



24.

25.



26.

27.

28.

29.

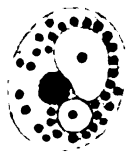
30.

31.

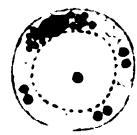
32.

33.

34.



35.



36.

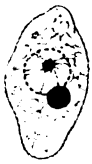
37.

38.

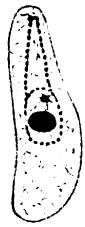
39.

40.

University Press, Oxford



41.

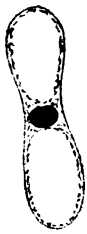


42.

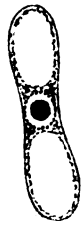
43.

44.

45.



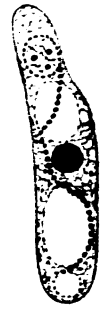
46.



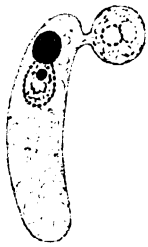
47.

48.

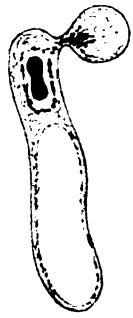
49.



50.



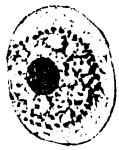
51.



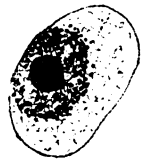
52.

53.

54.

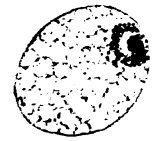


55.



56.

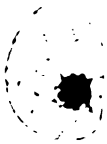
57.



58.

H.Wager del.

WAGER. — YEAST NUCLEUS.



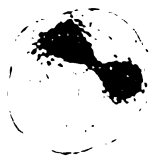
59.

60

61.

62.

63.



64.

65.

66.

67.

68.

69.

70.

71.

72.

73.

74

75.

76.

77.

78.

University Press Oxford

The Proteolytic Enzyme of Nepenthes (II).

BY

S. H. VINES.

IN the Annals of Botany for December, 1897, I published a paper on this subject, in which I adduced a considerable amount of evidence to prove that, contrary to the opinions of Dubois and Tischutkin, the pitcher-liquid of *Nepenthes* contains a proteolytic enzyme. Since writing that paper I have continued my observations, of which I now give some account by way of supplement.

Activity of Pitcher-Liquid.

I have nothing to add that would in any way modify my assertion that I have never failed to obtain digestion of fibrin by the liquid in a relatively short time, provided that the liquid was duly acidified. My new observations refer to the effect of exposure to high temperatures, of treatment with alkalies, and of filtration of the liquid, upon its digestive activity.

Heat. The following results will serve to illustrate the general effect of exposure to high temperatures. The method of experiment was to maintain the liquid for a given time at the required temperature, and then to institute a digestion-experiment, adding fibrin and the necessary acid; in nearly

[Annals of Botany, Vol. XII. No. XLVIII. December, 1898.]

every case there was a control digestion-experiment with unheated liquids:—

(March 1, '98.) Liquid heated to 70–80° C., for 15 minutes: digestion of fibrin (·05 grm.) not complete in 5 hours, though it eventually took place; control-experiment, digestion complete in $2\frac{3}{4}$ hours.

(March 3.) Liquid gradually heated from 60°–80° C., maintained at 80° C. for 5 minutes, then allowed to cool: total time of exposure to heat, 15 minutes; digestion not complete in 5 hours, but within 20 hours; control, digestion complete within 2 hours.

(March 8.) Liquid maintained at 80° C. for 15–20 minutes: digestion not complete until morning of the fourth day (March 11); control, digestion complete within 3 hours. In a subsequent experiment (May 17) the effect of treating the liquid to 80° C. for 20 minutes was less marked: in this case the time required by 10 cc. of the liquid to digest ·05 grm. of glycerin-fibrin was just 24 hours.

(March 15.) Liquid maintained at 78–83° C. for 30 minutes: digestion did not take place, although the experiment was continued for 4 days; in the control, digestion was complete in $1\frac{1}{2}$ hour. In a subsequent experiment (March 19) I found that liquid which had been kept at 80° C. for 30 minutes gave no indication of digestive action on fibrin although the experiment was prolonged for a week; it may be fairly concluded that the digestive power had been entirely destroyed. In the control, digestion was complete within 5 hours.

With regard to the action of a boiling temperature (100° C.), I was surprised to find, on several occasions, that liquid boiled for some seconds did not lose its digestive power, though the rate of digestion was made very much slower. It seems, in fact, that to entirely destroy digestive power, the liquid must be kept at 100° C. for an appreciable time, say 3–5 minutes.

Alkali. I have confined myself to the investigation of the action of sodium carbonate (Na_2CO_3) upon the digestive activity of the liquid. The method of experiment was to add to a quantity (usually 5 or 10 cc.) of the pitcher-liquid an amount of the solid salt requisite to produce the desired degree of alkalinity: the alkaline liquid was then placed in the incubator, and maintained for any required time at any

given temperature. After this treatment the liquid was neutralized, then acidified with HCl, and a digestion-experiment was made.

At an early stage it became apparent that the results of such experiments are dependent upon the three following factors:—(1) the degree of alkalinity; (2) the duration of the period of alkalinity; (3) the temperature maintained during the period of alkalinity.

I took as a starting-point the statement made in my paper of last year (p. 572), that the digestive activity of the liquid is destroyed by treatment with 5% Na_2CO_3 for 3 hours at a temperature of 35–40° C. I found, on repeating the experiment and prolonging it, that this conclusion is not accurate. In the original experiment the time allowed for digestion was 16½ hours, and in that period, it is true, no trace of digestive action could be detected: but in a subsequent experiment with a longer digestive period, evident signs of digestion were apparent in 20 hours, and digestion was completed in about 26 hours.

The following is a summary of the principal experiments made:—

1. *Temperature during period of alkalinity*, 35–38° C.

Treatment with 0·5% Na_2CO_3	for 30 minutes;	retards digestion.		
,, ,, 1%	,, ,, 2½	hours;	about doubles time of digestion.	
,, ,, 1%	,, ,, 17	,,	digestion much retarded.	
,, ,, 2%	,, ,, 6	,,	,, ,,	
,, ,, 4%	,, ,, 2	,,	digestion complete within 20 hours.	
,, ,, 5%	,, ,, 1	,,	digestion complete within 6 hours.	
,, ,, 5%	,, ,, 3	,,	digestion complete within 26 hours.	

It will be observed that, in all the foregoing experiments, digestion, though often much retarded, eventually took place. It still remained to ascertain under what conditions total destruction of digestive power could be effected by treatment

with Na_2CO_3. In view of the great retardation of digestion caused by treatment with 5 % Na_2CO_3 indicated in the foregoing table, I determined to experiment especially with this degree of alkalinity, but at a higher temperature: the temperature upon which I fixed was 50° C. The following are illustrative experiments:—

2. *Temperature during period of alkalinity*, 50° C.

(May 30.) Treatment with 5 % Na_2CO_3 for 45 min.; digestion much retarded.

(June 1.) ,, ,, ,, ,, 1½ hour; no digestion in 48 hours.

Hence it would appear that, in the second case, the digestive power had been totally destroyed.

The following are some further comparative experiments to determine more closely the degree and duration of alkalinity, at 50° C., necessary to destroy digestive action. In each case there was a control-tube, the liquid in which was heated to 50° C. for the same length of time as the others, but was not treated with Na_2CO_3, though diluted subsequently to the same extent as the others: the amount of fibrin used in the digestion was ·01 grm.

(*a*) Treatment with 5 % Na_2CO_3 for 1½ hour; no digestion in 6 days.

,, ,, 1 % ,, ,, ,, digestion complete in 4 days.

control; digestion complete in 3½ hours.

(*b*) ,, ,, 5 % ,, ,, 1½ hour; no digestion in 5 days.

,, ,, 1 % ,, ,, ,, no digestion in 5 days.

control; digestion complete in a few hours.

(*c*) ,, ,, 1 % ,, ,, 1 hour; no digestion in 4 days.

control; digestion complete in a few hours.

On comparing the results of *a*, *b*, and *c*, it would appear that treatment with 1 % Na_2CO_3 for one hour at a temperature of 50° C. is an approximate index to the stability of the enzyme.

Filtration. It occurred to me that some light might be thrown upon the bacterial explanation of the digestive activity of the pitcher-liquid by experiments with liquid which had passed through a Berkefeldt-filter. I found that liquid which has passed through such a filter has lost its acid reaction and its colouration. It still retains some digestive power, but is far less active than unfiltered liquid, the period of digestion being more than doubled.

This result might be made use of by the supporters of the bacterial explanation, as affording some sort of evidence in favour of that view; but even so, it would be far from conclusive. However, in order to test the value of this evidence I instituted some experiments with regard to the effect of filtration through the Berkefeldt-filter upon liquids containing pepsin and ptyalin. With regard to pepsin, I found that an unfiltered solution of glycerin-extract of pig's stomach digested ·05 grm. of fibrin in a quarter of an hour, whilst an equal quantity of the same solution after filtration required nearly 5½ hours to digest the same weight of fibrin. Similarly some diluted saliva caused the complete conversion of a small quantity of starch into sugar within a few minutes, whereas starch treated with filtered dilute of saliva continued to give more or less marked blue-reaction with iodine for four or five hours.

It is clear that solutions of pepsin and of ptyalin are affected by the Berkefeldt-filter in much the same way as is the pitcher-liquid of *Nepenthes*. If it be argued that the diminished activity of filtered pitcher-liquid is due to the removal of Bacteria, the same argument must equally apply to the solutions of pepsin and of ptyalin; but I do not think that any one would venture at present to attribute the action of gastric juice or of saliva to the presence of Bacteria. The obvious conclusion to be drawn from these experiments is

that enzymes are retained in a marked degree by the Berkefeldt-filter.

The Zymogen.

In my previous paper (p. 578) I stated that I had not succeeded in successfully repeating the experiments of 1877[1], which demonstrated the presence of a zymogen in the glandular tissue of the pitcher. The method which I adopted in 1877 was as follows: some pitchers were treated with dilute acetic acid (1 %) for 24 hours previously to the preparation of the glycerin-extract; comparative experiments between the glycerin-extracts prepared from pitchers so treated and the glycerin-extract prepared from pitchers gathered at the same time, but not treated with acid, showed that in every case the digestive power of the former was much greater than that of the latter, as indicated by the greater rapidity of digestion. In one experiment the acid-extract dissolved fibrin in 6 hours, whilst fibrin put to digest with the ordinary extract was but slightly attacked in that time. During the present year I have made some experiments with results confirmatory of those of 1877. They are as follows:—

June 2, '98. Took two unopened pitchers of *N. Mastersiana*; opened and washed out the pitchers; the pitcher-liquid strongly acid; cut up the glandular portions of the pitchers into small pieces; the whole weighed 8 grm.; divided into two halves *A* and *B*; *A* was rubbed up in a mortar with 20 cc. distilled water; *B* was rubbed up in a mortar with 20 cc. of ·25 % solution of HCl; both *A* and *B* were then placed for 45 minutes in the incubator at 50° C.; the liquid was then poured off from each, the substance dried somewhat with blotting-paper, and then rubbed up each with 20 cc. glycerin, to prepare glycerin-extracts *A* and *B*, and left to stand.

On June 10 the digestion-experiment was made: 5 cc. of each of the extracts were taken, and placed in a tube with 5 cc. of ·4 % HCl, together with ·01 grm. of fibrin; the tubes were put into the incubator (temperature about 37° C.) at 10.30 a.m. By 6.30 p.m. the fibrin in tube *B* (acid-extract) was completely digested, whereas

[1] Journ. Linn. Soc. xv.

that in tube *A* (neutral extract) did not disappear until over 48 hours later.

In another experiment of about the same date, in which, however, the pitcher-substance was treated with ·5 °/₀ acetic acid for about 24 hours at ordinary temperature (about 15° C.), the acid-extract digested more rapidly than the neutral, but the difference was not so marked as in the preceding case.

July 21, '98. Took two pitchers, one unopened, the other recently opened; cut up small; material divided into three parts of 2·7 grm. each: (1) was rubbed up at once with 20 cc. glycerin; (2) with 20 cc. of ·25 °/₀ HCl; and (3) with 20 cc. distilled water: (2) and (3) were placed in an incubator at 36° C. and were kept there till next day (18–20 hours), the liquid was filtered off from them, and they were each rubbed up with 20 cc. glycerin.

On Aug. 2 the digestion-experiment was made: 10 cc. of each extract were strained off through muslin; to each were added 10 cc. of ·4 °/₀ HCl, together with ·01 grm. fibrin, and all three were placed in the incubator (temperature 36° C.) to digest at 10.30 a.m. At 5 p.m. the fibrin in tube (2) (acid-extract) showed signs of digestion, and was almost entirely dissolved by 5 p.m. on the following day. The fibrin in tube (3) had all undergone solution by the evening of Aug. 5, whereas that in tube (1) still showed no sign of digestion.

A second digestion-experiment with the same extracts was made on Aug. 30, with essentially similar results, though the period of digestion was longer. The experiment commenced at 10.15 a.m., and at 5 p.m. no indication of digestion could be seen in any one of the three tubes. Next morning (Aug. 31) at 9 a.m. the fibrin in tube (2) (acid-extract) was seen to be attacked; the process of digestion continued slowly in this tube until it was complete (night of Sept. 2); the fibrin in the two tubes (1) and (3) underwent no perceptible change in this time.

The foregoing results suffice to show that, under certain circumstances, previous treatment with acid causes the glands of the pitcher to yield a more active glycerin-extract, or to yield an active extract when otherwise the extract would be inactive; and it can only be concluded that this must be due to the presence of a zymogen in the glands from which the enzyme is liberated on treatment with acid. However, I must

admit that, as pointed out in my paper of last year, I have by no means always succeeded in obtaining a more active extract as the result of treatment with acid; on the contrary, I have frequently found that previous treatment of the pitchers with acid diminished instead of increasing the activity of the glycerin-extract. I do not regard these apparent contradictions as wholly attributable to various conditions of the pitchers, for I have obtained sometimes quite opposite results with pitchers as nearly as possible of the same age, and sometimes quite similar results with pitchers of different ages (*e.g.* opened and unopened). On the contrary, my results seem to show that the differences in the activity presented by the various acid-extracts are due rather to the mode of treatment of the pitcher-material. It would appear that the most effectual mode of decomposing the zymogen is to act upon the tissue with acid for a short time at a relatively high temperature (see example of June 2–10). More prolonged treatment at a lower temperature (say 35° C.) would seem to cause not only the liberation of the enzyme, but also its extraction from the glands in connexion with the digestion of the pitcher-tissue itself.

I may just point out in conclusion that the marked acidity of the liquid in the unopened pitcher is no doubt to be connected with its high digestive activity; whilst the acid is useless for digestive purposes until the opening of the pitcher, it is probably of importance in that it acts upon the zymogen, liberating the enzyme.

The Products of Digestion.

In my paper of last year I pointed out that the chief proteid product of digestion was a substance closely resembling deutero-albumose, and I stated further that I had failed to detect the presence of a true peptone, that is, of a proteid which is not precipitated on saturation with ammonium sulphate. I have, however, since detected the presence of peptone, though in relatively small quantity, among the products of the digestion of fibrin by the pitcher-liquid.

The clue to the matter was found on this wise. The method which I had followed (see my paper of Dec. '97, p. 579) in examining the products of digestion involved the precipitation of these substances by filtration into excess of alcohol. Whilst investigating the nature of the ultimate products, I evaporated a considerable quantity of the alcohol which had been used for precipitation, and this left a dark brown syrupy residue. Some of this residue, dissolved in a small quantity of distilled water, formed a brownish solution, giving no precipitate on boiling, but good xanthoproteic and biuret reactions. A portion of this solution was put to saturate with ammonium sulphate and gave a dense precipitate which brought down with it all the colouring-matter; the clear, colourless liquid obtained on filtration still gave strong xanthoproteic reaction, and continued to do so after continued saturation for two days longer. Another portion of the brown solution was put to dialyze, and within twenty-four hours the dialysate gave strong xanthoproteic reaction; on saturating the dialysate with ammonium sulphate, there was a precipitate, the filtrate from which still gave the xanthoproteic reaction, and continued to do so on further saturation. These observations indicated the presence of peptone without, however, absolutely establishing it; for it might be the case that the precipitation of the deutero-albumose by means of ammonium sulphate had been incomplete, and that the proteid reactions were due to this substance rather than to peptone.

It became necessary, therefore, to employ some method by which the separation of deutero-albumose and peptone could be certainly effected. Fortunately I applied for advice to Mr. Ramsden, Fellow of Pembroke College, who has an intimate knowledge of the chemistry of proteids, and he kindly directed me to a paper by Kühne[1] on this very point. Kühne's method consists in saturating the neutralized digestion-liquid with ammonium sulphate when boiling. After

[1] Zeitschrift für Biologie, 1892 (Erfahrungen üb. Albumose und Pepton). It may be asserted that, until the appearance of this paper, no true peptone had been obtained free from albumose.

cooling and filtering, the liquid is again heated and made alkaline with ammonia, and is then again saturated with ammonium sulphate. It is once more cooled and filtered, then boiled to drive off the ammonia, again saturated with ammonium sulphate whilst boiling, and made acid with acetic acid. The excess of ammonium sulphate is then got rid of by adding alcohol to the liquid; most of the ammonium sulphate is precipitated, and the supernatant liquid holds in solution whatever peptone is present, which may be ultimately obtained by repeated treatment with alcohol and decantation of the supernatant liquid.

With this method at my disposal, I had no difficulty in demonstrating the presence of peptone in the digestion-liquids, though it was necessary to concentrate these liquids before proceeding to test them. Mr. Ramsden also found peptone in a quantity of digestion-products precipitated by alcohol, which he was good enough to examine for me.

I have nothing to add with regard to the other products of digestion, beyond the fact that I have been able to confirm my statement that leucin is one of them.

Conclusion.

1. The experiments relating to the action of high temperatures and of alkalies upon the enzyme confirm the statement made in my paper of last year with regard to its great stability; in fact, it would appear that it is the most stable of all known proteolytic enzymes. Whilst its activity can easily be much diminished by exposure to high temperature or treatment with an alkali, it still retains a sort of residual digestive power which asserts itself in very slow and prolonged digestion, and which can only be destroyed by relatively strong measures.

2. It may, I think, be fairly concluded from the facts given in this paper, in conjunction with those which I published in 1877, that the enzyme is derived from a zymogen present in the gland-cells.

3. The discovery of true peptone among the products of digestion facilitates the classification of the enzyme. Green[1] has found that the proteolytic ferment present in germinating seeds acts in an acid medium, producing a relatively large quantity of albumose together with peptone, leucin, and tyrosin; that it is in fact a *tryptic* ferment, differing mainly from the trypsin of the pancreatic juice in requiring an acid medium for its digestive action. In all these respects (though I have not made out the production of tyrosin) the proteolytic enzyme of *Nepenthes*-pitcher closely resembles that of the germinating seed; but it is much more rapid and energetic in its action, and apparently more stable in its nature. These two proteolytic enzymes are distinguished, by their activity in an acid medium, from those, such as papaïn[2] and the enzyme in the fruit of *Cucumis utilissimus*[3], which are most active in a faintly alkaline medium. It is a remarkable fact that, whatever may be the reaction of the medium in which they can work, all these enzymes are essentially tryptic in their mode of action; in fact it is not improbable that this may be a characteristic feature of all vegetable proteolytic enzymes whatsoever.

[1] Phil. Trans., 1887.
[2] See Martin, in Journ. of Physiol., V, 1884, and VI, 1885.
[3] See Green, in Annals of Botany, VI, 1892.

NOTES [1].

CHANGES IN THE SEX OF WILLOWS.—In the genus *Salix* flowers of both sexes are occasionally present in the same catkin, and one sometimes finds that the sexual organs are intermediate in structure between stamens and carpels. By using the published records, and by availing myself of the large accumulation of material for study in the Herbaria at Kew, Cambridge, in the British Museum, and at the Jardin des Plantes, Paris, I have gathered together a number of facts which may be of interest.

Firstly, it is obvious that these abnormalities, though widely distributed in the species of *Salix*, are much more common in some sections than in others. The section *Capreae* yields by far the greatest number; and second to it comes the section *Fragiles*. In dwarf willows they seem to be very rare, and in the section *Glaciales* I have only found one abnormal catkin.

Secondly, we notice that, though the two-staminal willows yield most freely these abnormalities, those in which the male flowers possess more than two stamens sometimes show them. I can instance *S. pentandra* and *S. humboldtiana*.

That the male organs or the female organs are produced from the same rudiments is extremely probable; and in the normal *Salix* we have an unisexual flower, which cannot, as in most Phanerogams, be shown to have had an origin from a hermaphrodite flower by abortion of one sex. In these abnormal willows, while we readily follow the change of the two stamens of one of the *Capreae* or *Purpureae* into the two carpels, it is not so easy to say what happens when five or more stamens have to be replaced by two carpels.

[1] These Notes are abstracts of papers read before Section K of the British Association, at the Bristol Meeting, September, 1898.

Lastly, of the several theories thus far proposed to account for the occurrence of the abnormalities, none is capable of wide application.

Sometimes the abnormalities reappear year after year; sometimes they prove inconstant. Had we a fuller knowledge, some explanation, partial or complete, might be forthcoming; for frequently, both in their distribution in the catkin and on the branch, the changes in sex show a tendency to arrangement. At times the male is above the female; at times the reverse is the case. Rarely there are three or four belts of flowers on one catkin, male succeeding female, and female male, in definite order.

ROYAL GARDENS, KEW. I. H. BURKILL.

THE ANATOMY OF THE STEM OF SPECIES OF LYCOPODIUM.—Ten species of *Lycopodium* have been examined; among these two types may be distinguished.

1. Type of *L. clavatum* (L.). The oval stelic arrangement is marked by a considerable amount of xylem, broken up into patches by bands of phloem. Centrally these bands are strap-shaped, but at the ends of the long axes the areas of phloem are external, and occur as curved and flattened wedges. Large cells without contents, sieve-tubes, appear in the centre of the strap-shaped bands. Protophloems and protoxylems are external, forming a continuous ring, as figured by Hofmeister; so that, using De Bary's terminology, the arrangement of the bundles is radial. Pericyclic and the so-called endodermal cells occur in concentric zones, 1–3 cells broad. The former swell up, especially in glycerine or glycerine-jelly; the latter are generally considerably lignified. The cells of the cortex lying just external to the endodermal cells are thickened and lignified, forming a third concentric zone several cells deep. To this type conform *L. alpinum* (L.), *L. Phlegmaria* (L.), *L. dendroides* (?), and *L. cernuum* (L.).

2. Type of *L. squarrosum*. The type which contrasts most markedly with the former is found in *L. squarrosum* (Forst.), *L. dichotomum* (Jacq.), and *L. nummularifolium* (Blume). The phloems occur as islands in the sea of xylem, or as inserted peninsulas. The phloems are centrally built up, with the apparent sieve-tubes in the centre. Protoxylems are well marked, and lie externally, but protophloems are not to be distinguished. Endodermal cells and pericycle are found as in the previous type. The sclerenchymatous sheath is wanting, or very slightly developed.

The two remaining species, *L. Dalhousieanum* (Spring) and *L. Selago* (L.), are, to some degree, intermediate types. The phloem in *L. Dalhousieanum* shows both types, strap-shaped and centric. In the branches the structure becomes simpler. There are two narrow strips of xylem, with an intermediate strip of phloem, so that a prominent row of sieve-tubes occupies the very centre of the stelic cylinder. *L. Selago* in its structure is modified on that of *L. clavatum*. An interesting feature of *L. Selago* and *L. squarrosum* is the occurrence of root-structures running through the stem. These consist of steles containing a crescent-shaped mass of xylem, with protoxylems towards each tip, while the concave portion is filled up with phloem. A characteristic sclerenchymatous sheath surrounds the stele. In *L. Selago* these root-structures are found even above the point where the stem branches, but in *L. squarrosum* they have fused with the central cylinder before branching occurs.

C. E. JONES.

UNIVERSITY COLLEGE, LIVERPOOL.

REPRODUCTION IN DICTYOTA DICHOTOMA.—1. *Dictyota* is an annual. In this country it germinates during the summer, remains small during the winter, grows very rapidly in June, and begins to form its reproductive cells in July.

2. The tetraspores are produced throughout the season, and all stages may be found together on the same plant. The sexual cells, however, show a remarkable periodicity. The formation, maturation, and liberation of each crop occupies a fortnight, the interval between two spring-tides. The sori are formed during neap-tides, and the cells are liberated during or immediately after the highest spring-tides.

3. When liberated the oospheres are not invested with walls. In this condition they strongly attract the antherozoids, become fertilized, and at once start germinating. The plantlets are similar to those figured by Thuret as resulting from the germination of the tetraspores.

4. If not fertilized the eggs lose the power of attracting antherozoids, they form walls, and, as already described by Thuret and Bornet, they germinate parthenogenetically. After one or a few divisions, sometimes accompanied by formation of a rhizoid-rudiment, the process stops and the plantlets die.

5. Towards the close of the season some sori fail to mature within

the usual period, and the crops become less regular; the same effect is brought about during very cloudy and cold summers.

6. The same conditions bring about sterilization of certain of the sexual cells. Thus, patches of cells within the antheridial sori fail to divide. Cells at the margins of female sori remain barren, so that the usually borderless sori acquire partial or even complete borders.

7. There are strong reasons for concluding that the factor which determines the maturation and liberation of the sexual cells, and the fertilization of the oospheres, is the amount of the illumination to which the plants are subjected.

8. The cytology of the reproductive cells will be described as far as it has been made out.

UNIVERSITY COLLEGE, BANGOR. J. LLOYD WILLIAMS.

CHANGES IN THE GLAND-CELLS OF DROSERA PRODUCED BY VARIOUS FOOD-MATERIALS.—The work is an extension of that previously undertaken by the authoress, an account of which has already appeared in 'Quart. Micro. Journ.,' vol. xxxix.

In the experiments now described, leaves were fed with egg-albumin, globulin, peptone, fibrin, milk, nuclein, nucleic acid, and calcium phosphate, the histological changes in the gland-cells being noted in each case.

The results to be described were obtained with fixing-fluids, widely differing in their chemical constitution.

Egg-albumin. The basophil cytoplasm becomes pink, and is reduced in twenty to thirty hours to a mere vestige. After two days it commences to recuperate, and ultimately becomes again basophil. The changes in the nucleus comprise—(1) those of the nuclear chromosomes, (2) those of the nuclear plasm, and (3) those of the nucleoli. In the resting-cell the nuclear chromatin is scanty, but immediately after feeding it commences to increase, till in twenty to thirty hours large segments are formed as in mitosis. During recuperation the segments again diminish. The eosinophil nucleoli are large in the resting-cell; they diminish after feeding in direct proportion to the increase of the basophil chromatin, and finally enlarge when the chromatin-segments diminish.

Peptone is absorbed much more rapidly than egg-albumin, and produces in one hour changes similar to those effected by egg-albumin in twenty to thirty hours.

Globulin also produces changes in twenty-four hours, but to a less marked degree than egg-albumin. Food passes into the tentacle between the lateral walls of the cells, and secretory products pass through the apical walls, thus producing an appearance of striae in the food which is in contact with the tentacles.

Fibrin is digested slowly, and changes similar to but generally less pronounced than with egg-albumin are seen.

Milk is absorbed rapidly and completely. The morphological changes are less marked than with any of the above-mentioned foods. The cell-plasm remains basophil throughout.

Nuclein produces almost no effect; the tentacles do not bend in, and do not secrete more copiously than before. No cytological changes are produced except very slight vacuolation of the cell-plasm. All the colour reactions are the same as those of controls.

Nucleic acid produced rapid bending in of the tentacles, and extremely copious secretion. The leaves reopen in one to three days, and although the quantity of nucleic acid given is not perceptibly diminished, there are great histological changes, consisting in an almost complete disappearance of the cytoplasm (which remains basophil throughout), and of the nucleoplasm. The basophil chromatin-segments remain unaltered.

Calcium phosphate produces appearances very similar to those after feeding with egg-albumin, but the cytoplasm remains basophil.

Control leaves, after the application of all the above substances, reopened in a perfectly healthy condition, as determined by their naked-eye appearances while living, and their microscopic structure after fixing by different methods.

LILY H. HUIE.

A POTATO-DISEASE.—I have for some time past had occasion to recognize here and there, in various parts of England, a potato-disease which is not due to *Phytophthora*, and which has often been ascribed to bacteria. During the past two years my attention has been especially directed to testing its bacterial origin, and I am convinced that it is not due to bacteria, but to a true hyphomycetous fungus.

Without going so far as to say there is no bacterial disease of the potato, I wish to express the conviction that the alleged cases of such

lately published are not convincing, and that a tendency exists to draw conclusions from imperfect evidence.

I shall show that the way into the tuber is prepared for bacteria by fungus-hyphae, and the open passages of destroyed vascular bundles afford them ample space. The disease I have studied has appeared in a more or less epidemic form at least twice in my experience: it was very common two years ago, and this year has been abundant in various parts of England. In a subsequent publication I shall show that it is common and widespread, and even known in some countries, though not adequately recognized.

Symptoms.—The shoots turn yellow and die prematurely during the summer, and before the tubers are anything like full. The disease *starts from below* and *not from the leaves*. The roots are few and poor, and soon rot away. The tubers are few, do not mature, and often rot in the ground. The leaves turn yellow and wither on the stems, with the symptoms of *premature wilting*, and often remain long hanging on the yellowing, glassy-looking, but still living stems.

In very mild cases these symptoms are not obvious, and supervene slowly, and the case may be complicated by the coexistence of *Phytophthora*. In very severe cases, on the other hand, especially in wet situations, the stems and roots may be all rotten by the end of July, and casual observation may ascribe the damage to *Phytophthora* entirely. In ordinary cases, again, it is easy to suppose the damage due to some insect attack, or to drought.

In advanced stages of the disease the stems either dry up to brown sticks, or putrefy on the wet ground; very often bacteria have gained access to the tissues at a comparatively early stage.

Microscopic Appearances.—Sections across the lower parts of the attacked stems show one, two, or more of the vascular bundles yellowish-brown—visible even without a lens—and the principal vessels of these contain branched, septate hyphae. In several cases I have traced these hyphae through every internode of the stem, into the petioles of the still hanging leaves, into the young lateral shoots, throughout the roots and subterranean rhizomes, and *up to and even just into the tubers*. In two cases I have done this in one and the same potato-plant, and so have no longer any hesitation in ascribing the disease to this fungus, the morphological features of which will be described in a subsequent paper. In advanced cases the brown vessels are stopped with a yellowish gum-like substance. Tyloses are common in the vessels of

the root. Those tubers which are not attacked while still very young, but which have already begun to fill with starch, may offer considerable resistance to the invasion of the fungus; but eventually the vascular strands diverging from the point of attachment to the rhizome exhibit the tell-tale foxy-red or yellowish-brown colour, and in many cases the ripened tubers are to all appearance sound, except for *microscopic reddish spots just at the points of entry of these bundles.*

During the winter the stored potatoes, with the fungus thus just lurking in them at the morphological base (the so-called heel) of the tuber, may undergo little change to all appearance *if gathered and stored dry.*

But if wet, various kinds of rot may supervene, owing to the *subsequent invasion* of various micrococci, bacteria, fungi, &c., following the lines of weakness opened up by the fungus in question, and living as saprophytes on the stored reserves.

In some cases even apparently dry tubers may undergo a curious rot—dry-rot—owing to the ravages of a particular bacterium or mould, perhaps more than one, which finds sufficient moisture for its purposes.

The principal point is that the fungus I have especially studied leads the way for these purely saprophytic anaërobic and aërobic forms into the tuber: once in the mature tuber, its progress is necessarily slow until the reserves move in the spring.

During the past winter I gave to Miss Dawson, who is working at such subjects in my laboratory, some of the tubers saved from plants attacked with this disease, to investigate the various fungal forms lurking in the diseased tubers. Her investigations are not yet completed, but enough has been accomplished to convince us that after the fungus in question has opened up the way into the tuber, all sorts of bacteria and fungi can make their way down the destroyed vascular strands, and reappear in spring, when the tubers are replanted.

But this is not all. The evidence shows that the fungus in question, once in the tuber, leads a dormant life during the early part of the winter, but gradually invades the new sprouts as they slowly appear in the early spring, and that *the parasite is actually replanted* by the farmer or gardener, when restocking the ground, *in his new 'sets.'*

If we reflect that the tuber is really a bud, there is nothing especially strange in this phenomenon; the fungus enters the base of the bud in autumn, and takes some months to traverse its dormant tissues during

the winter and spring. A spotted tuber may give rise to some healthy and some diseased sprouts, according to the tracks of the fungus.

A curious phenomenon was observed in some potato-plants very badly attacked by this disease this summer. In some of the badly diseased young shoots, quantities of beautifully developed *cubical proteid crystals* (crystalloids) were observed in the parenchyma of the pitch and cortex. It is due to Mr. W. G. P. Ellis to point out that he was the first to see these in some sections he was kindly cutting for me of this batch of specimens. On going further into the matter I find such crystalloids have been seen by Heinricher in the shoots of a diseased potato[1], but he did not give any account of the disease itself.

I find these crystals are not uncommon in the still green bases of the petioles of the withered leaves hanging on the diseased shoots, though they do not always occur.

I ascribe their formation to the accumulation of proteids in the leaves, while still living and active, from which the passages of transference at the nodes of the stem have been cut off by the fungus; just as the eventual withering of the leaves is due to the blocking of their water-conduits when all the vessels are stopped up.

At the same time, the attempts I have made to induce the formation of these crystalloids artificially have failed so far.

Neither ringing, nor ringing combined with destruction of the pith with a hot skewer—to destroy the internal phloëm—has given satisfactory results as yet, though the leaves of healthy plants withstand this drastic procedure much better than might be supposed.

Here again I must reserve further particulars for the fuller paper.

In conclusion, it is evident that the efforts of the potato-grower must be directed to the selection of sound sets, and to the careful preparation of his ground. I hope to show later that it is a fatal procedure, even with sound sprouts, to allow the young shoots to lie in contact with raw manures, as it is *viâ* wounds and small rotting spots at and near the collar that new infections occur. The same arguments apply to wet soils and situations, and the disease is particularly apt to increase when wet and cold weather supervenes on the early growths.

H. MARSHALL WARD, Cambridge.

[1] Ber. d. deutsch. bot. Ges., 1891.

PENICILLIUM AS A WOOD-DESTROYING FUNGUS.—Spores from pure cultures of *Penicillium* were sown on sterilized blocks of spruce-wood, cut in March, and were found to grow freely and develop large crops of spores on normal conidiophores. Sections of the infected wood showed that the hyphae of the mould entered the starch-bearing cells of the medullary rays of the sap-wood and consumed the whole of the starch. The resin was untouched. In culture three months old the hyphae were to be seen deep in the substance of the wood passing from tracheide to tracheide *viâ* the bordered pits. Control sections, not infected and kept side by side with the above, contained abundance of starch, and no trace of hyphae could be detected in them.

The observation appears of interest in several connexions. *Penicillium* is one of our commonest moulds, and undoubtedly plays a part in the reduction of plant *débris* to soil-constituents; how far it can itself initiate the destruction of true wood, or how far it merely follows on the ravages of other fungi, bacteria, &c., is unknown. There are strong grounds for believing that it destroys the oak of casks, &c., but since these are impregnated with food-materials this is not very surprising. Trabut[1] has shown that *Penicillium* will grow in solutions containing 2–9·5 per cent. of $CuSO_4$, and other evidence exists showing how remarkably resistant this mould is, and how little organic matter it needs for life.

Dubois[2] showed that *Penicillium*, or a closely-allied form, not only lives in strong solutions of copper, neutralized with ammonia, but will erode metallic copper and bronze if transplanted thereon.

Jönssen[3] found *Penicillium* living in one-tenth normal sulphuric acid solution, and gives some interesting facts regarding the sulphur-containing oil-drops in its protoplasm, and other statements concerning oil in this fungus occur in the works of De Bary, Brefeld, Pfeffer, &c.

Gerard[4] gives proof that *Penicillium* can liberate butyric acid from mono-butyrine, and evidence that this is due to its power of forming a *lipase* or fat-splitting enzyme.

Lesage[5] gives striking instances of the resistance to externa

[1] Bull. de la Soc. Bot. de Fr., xlii, 1895, 1.
[2] Comp. Rend., 1890, cxi, p. 655.
[3] Bot. Centr., xxxvii, 1889, p. 201.
[4] Bull. de la Soc. Mycol. de Fr., xiii, 1897, p. 182.
[5] Ann. des Sc. Nat., Sér. 8, T. i, 1895, p. 309.

influences shown by the spores on germination. Not only will they germinate and live for some time in water, and under almost anaërobic conditions, but he found them germinating in 26·5 per cent. solutions of common salt; 30 per cent. solutions were too much for them, however. He states also that the vapours of cedar-oil, iodoform, napthalin, camphor, and patchouli do not prevent germination; though those of clove-oil, ether, alcohol, chloroform, and acetic acid prevent it. The maximum for alcohol was somewhere between 4·2 and 6·2 per cent. In acetic acid they germinated in twenty-four days in solutions of 1 : 256, but failed to do so in solutions of 1 : 64, whereas in HCl they germinated in two days in 1 : 4 solutions.

As regards temperatures, it is well known how resistant the spores are. A striking instance of the hardships the mycelium can undergo is given by Woronin[1]: he found *Penicillium* vegetating on the melting snow, where the temperature at night fell below 0° C.

Bourquelot[2] found invertase, maltase, trehalase, emulsin, inulase, diastase, and trypsin in the allied *Aspergillus*, and pointed out how suggestive this is in explaining the ubiquity of this mould. Probably *Penicillium* is equally rich in capacity for enzyme-production.

Miyoshi[3] showed that *Penicillium* can bore through cellulose membranes, and no doubt similar chemotactic phenomena are concerned in the piercing of wood-elements by the hyphae.

It certainly looks as if *Penicillium* may be a much more active organism in initiating and carrying on the destruction of wood than has hitherto been supposed, and that it is not merely a hanger-on or follower of more powerful wood-destroying fungi. It is also, doubtless, very independent of antiseptics.

H. MARSHALL WARD, Cambridge.

A METHOD OF OBTAINING MATERIAL FOR ILLUSTRATING SMUT IN BARLEY.—By sowing soaked, skinned barley that had been plentifully covered with *Ustilago* spores a supply of smutted barley may be ensured, and in such material it is easy to trace out the spore formation.

Hand-sections of the ear when about ⅝ inch long showed the

[1] Arb. d. St. Petersb. Naturf.-Ver., B. xx, p. 31.
[2] Bull. Soc. Mycol., 1893, p. 231.
[3] Bot. Zeit., 1894, H. 1.

mycelium at the growing-points of the flower shoots, and in such sections the mycelium, at first intercellular, could readily be found becoming intracellular and of much greater diameter. Branches became very numerous, and in the hyphae and branches spores were formed. Towards the central parts spore-clusters were too dense for examination, but nearer the epidermis the branching and arrangement of the sporogenous hyphae could more easily be made out; and the teasing of the lateral flowers of each notch of the rachis was often more successful than if the central—and *only* flower of the ordinary ear—were taken. Sections were mounted in water, and some in 1 per cent. KOH, and it is but fair to say that such treatment has failed to show any septation of the hyphae as a preliminary to spore-formation. Material for microtome-sections was prepared as follows:—The leaves of a barley-shoot were stripped down so as to expose the apparently highest node, and the part an inch or two above this was cut off; then by a series of successively lower horizontal cuts the youngest leaves were removed until in the space they enclosed the tips of the awns or ear were seen; then a cut was made through the node, and the removed ear was placed in Flemming's or Rath's solution for fixing, the ear thus being, for a very few seconds only, between plant and reagent.

If a smutted ear be removed and kept floating on water, its spores continue to develop, and in several cases they matured first in the awn. It was by no means uncommon, on teasing out young fruits from such an ear, to find that the spores had germinated.

I have not yet made similar observations for *Tilletia* as my bunted wheat was less forward than my smutted barley, but I am satisfied that by this method of working class material for illustrating Bunt and Smut may easily be obtained.

W. G. P. ELLIS, Cambridge.

STRUCTURE OF THE YEAST-CELL.—A study of the cells of *Saccharomyces Cerevisiae* has led me to the following conclusions, part of which merely confirm former researches: (1) A relatively large nuclear body exists in each adult cell. (2) Young cells contain no such body; a little later the old nuclear body divides, and one of its two daughters wanders through the narrow connecting-channel into the young cell. (3) After the division is complete, the two cells are still kept together by a mucilaginous neck-shaped pedicel, which

appears not to have been noticed hitherto. It may persist or not, thus explaining the occurrence of cell-chains or of isolated cells in different races of Yeast. (4) Carbohydrates are stored up in Yeast in the form of glycogen, which accumulates or disappears from the vacuoles very rapidly, according to conditions of nutrition and growth. The colour given by a known quantity of iodine-solution to a known amount of Yeast-culture shows these variations most sharply. The change of tint by heat after iodine-action, and the destruction of the intracellular glycogen by saliva, also give very clear results.

L. ERRERA, Brussels.

OSMOTIC OPTIMUM AND MEASUREMENTS.—Recent researches made by Dr. F. Van Rysselberghe in the Botanical Institute of Brussels have shown that vegetable cells generally answer an *osmotic stimulus* by an appropriate *osmotic reaction*, and that the relation between stimulus and reaction follows, within wide limits, the 'law of Weber.' Hence results the possibility of predicting the existence and value of an *osmotic optimum*.

Let n be the normal osmotic pressure in a given cell;

x the osmotic pressure of an external solution applied as stimulus;

R the reaction, i.e. the change in the osmotic pressure of the cell in response to this stimulus. Then one has, according to Weber's law:

$$R = c \log \frac{x}{s} \quad (c \text{ and } s \text{ being constants}).$$

The total value of the osmotic pressure in the cell is of course $R+n$, and its excess over the pressure of the surrounding solution is,

$$y = R+n-x,$$

$$\text{or } y = c \log \frac{x}{s} + n - x.$$

It is easy to find by differentiation that this excess has a maximum value when $x = c \log e$ (e being the basis of the Napierian logarithms $= 2{,}7182818\ldots$).

Experiments made with *Tradescantia*, *Symphoricarpus*, *Allium*, *Elodea*, *Spirogyra*, agree most satisfactorily with these theoretical results.

Additional interest arises from the fact that these values of x really

correspond to *optimal* solutions, in which the cells live longer than in any other.

The investigations just alluded to have proved that de Vries' constant *isotonic coefficients*, excellent as they are for a first approximation, are not sufficiently exact for more minute experiments. Here it is advisable to use, instead of them, the coefficients of electric conductivity, which vary slightly with the concentration of the solution.

Thus, osmotic pressures are not strictly proportional to the concentration of the plasmolysing solutions, and these pressures ought no more to be expressed in molecule-grams of NO_3K, as is now generally done. The use of *an atmosphere* as unit, though better, is also objectionable, as it varies from one place to another.

I would therefore suggest to adopt the C.G.S. unit of pressure, viz. 1 dyne per sq. cm., or rather (to avoid useless decimals) 1 *myriadyne per sq. cm.*, i.e. the pressure of 10,000 dynes per sq. cm., the dyne being the force which gives the mass of 1 gram in 1 second an acceleration of 1 cm. per second.

This unit is roughly equal to $\frac{1}{100}$ atmosphere; we found it to be very convenient for all sorts of osmotic calculations.

L. ERRERA, Brussels.

THE FORM OF THE PROTOPLASMIC BODY IN CERTAIN FLORIDEAE.—In *Ceramium rubrum* and other species a strong strand of protoplasm runs along the axial cells from pit to pit. In this strand the nucleus is occasionally suspended; more often it lies over the pit at the base of the strand.

In *Dasya coccinea* the branches of limited growth run out into pointed uncorticated filaments, the cells of which are large. Across the vacuole of these cells, running from pit to pit, occurs a thread of protoplasm much more delicate than the corresponding structure in *Ceramium*.

In *Callithamnion byssoides* threads of protoplasm radiate from a cushion lying over the pit and end blindly on the vacuole. These threads are in incessant movement, swinging over, bending on themselves, and extending or retracting. All these phenomena point to the great physiological importance of the pit-communication between cell and cell.

R. W. PHILLIPS.

University College, Bangor.

ALTERNATION OF GENERATIONS IN THE THALLOPHYTES.—Since the pioneering investigations of Hofmeister, it has been generally recognized in Botany that the Archegoniatae are characterized by a definite form of alternation of generations. It consists in the regular alternation of a part which bears the sexual organs—the gametophyte—and of a non-sexual part which produces the spores—the sporophyte. An essential difference separates the two divisions of the Archegoniatae. In the Pteridophyta the gametophyte is a delicate, short-lived, thalloid structure, the sporophyte is a well-developed, leafy plant. In the Bryophyta, however, the gametophyte appears as a leafy plant, while the sporophyte is represented by a leafless stalked capsule, which lives as it were parasitically on the gametophyte.

In sharp contrast to the harmonious unanimity which has hitherto been the rule in Botany as regards this alternation of generations in the Archegoniatae, is the lively contest of contradictory views as to the alternation of generations in the lower plants. With regard to these the question arises, first, whether a regular alternation of definitely characterized generations is to be observed; and secondly, what in that case is the connexion between this alternation, should it turn out to be a fact, with that of the Archegoniatae. I shall not deal exhaustively here with the many different opinions on this question; I shall briefly touch upon those views only which are important in point of principle.

The first clear carrying out of the idea of a regular alternation of generations in the Thallophytes is in the Text-book of Sachs (1874), who there endeavoured to make the course of development of Algae and Fungi fit with that which holds in the Mosses. The life of *Vaucheria*, *Mucor*, an Ascomycete, or one of the Florideae, is divided according to Sachs into two sharply separated parts, of which one is characterized by the appearance of sexual organs, the other by the spore-bearing tissue which springs from the fertilized ovum. Thus the mycelium of a *Mucor* which bears the sexual organs, the thallus of *Vaucheria*, or of the Florideae, represent what we now term the gametophyte; the fruit-body of Ascomycetes or of Florideae, the zygospore of *Mucor*, the oospore of *Vaucheria*, represent the second non-sexual generation—the sporophyte. The alternation of generations of the Thallophytes is therefore, according to Sachs, essentially similar to that in the Archegoniatae. The

propagation by zoospores, conidia, &c., corresponds to the propagation by buds in the Mosses and Ferns, and is not taken into account as regards the actual alternation of generations.

Pringsheim takes up a quite opposite point of view (1876, 1878). According to his opinion the fruit of the Ascomycetes and Florideae has not the value of a special generation, but is only to be regarded as a part of the mother-plant sexually influenced. The true alternation of generations of the Thallophytes consists, according to Pringsheim, in the regular succession of independent so-called neutral generations, having non-sexual propagation, and a single sexual generation. Thus, zoospore-forming generations of *Vaucheria*, or *Oedogonium*, alternate with a generation which bears the sexual organs. Both kinds of generations are of essentially similar structure; they are distinguished by the form of their propagation. Only the first generation, which springs from the fertilized ovum, has often properties which differ from those which follow, e.g. in *Coleochaete*. In the Mosses this first non-sexual generation is much more sharply characterized; it is developed as the sporogonium, and is the only neutral generation; it differs from the sexual generation only by the scanty development of the vegetative part.

While the views of Sachs on the one hand, and of Pringsheim on the other, were showing some tendency to spread, other views appeared in opposition, now to one, and now to the other. Vines (1879) held that most of the Thallophytes have no alternation of generations at all, since their mode of propagation, whether sexual or non-sexual, is directly dependent upon external conditions; that a definite alternation of generations, comparable to that of the Mosses, is only found in *Coleochaete* and *Chara*. Celakovsky (1877), however, was more in accordance with Pringsheim in his conception of the Thallophytes, for like him he accepts an alternation of neutral and sexual generations. Celakovsky designates this alternation of generations as homologous, since the successive generations are equivalent to one another. Celakovsky opposes Pringsheim in his conception of the alternation of generations in the Archegoniatae, which he designates as the antithetic. Here the two alternating generations are not homologous, but essentially different; the non-sexual generation has also phylogenetically nothing to do with the neutral generations of the Thallophytes. This conception of Celakovsky was at first neglected, but was taken up again by Bower

(1890), and put on a footing of detail. Bower holds that the antithetic alternation came about by the intercalation of the non-sexual generation, the sporophyte, as a quite new development between two gametophytes. This interpolation of a special sporophyte probably took place in the alga-like ancestors of the Archegoniatae, as they passed from a life in water to a life upon the land. In the series of the Thallophytes there are, in addition to the homologous alternation of generations, more or less advanced beginnings of an antithetic alternation, as for instance in *Coleochaete*, the Ascomycetes, and Florideae.

All these various conceptions of alternation of generations in the Thallophytes rest on morphological comparison of the hitherto known facts of the life-history, while still very little was known of the behaviour of these organisms in open nature, or in long-continued cultures. Yet by such observations only is it possible to decide whether an alternation of generations can be proved at all, and how the influence of the outer world, so often assumed, really affects the course of life of the Thallophytes. These questions were the point of departure for my investigations on the conditions of propagation in Thallophytes. The investigation had to be extended in two directions: in the first place, it was necessary to decide whether there is a regular alternation of free and independent generations; secondly, whether a non-sexual generation characterized by special qualities arises of inner necessity from the fertilized ovum.

The first question receives its answer, according to the results of my investigations, that no regular alternation of neutral and sexual generations exists in any of the Thallophytes which have been tested. They possess two or several kinds of propagation, each of which is directly dependent upon quite definite external conditions. If we take any vegetative stage we please, a filament of a *Vaucheria*, an *Oedogonium*, a piece of mycelium of a *Sporodinia* or *Ascoidea*, there are then present in each part the specific potentialities of sexual and non-sexual propagation. In open nature the fortuitous conditions determine which of the potentialities is developed, and how the modes of propagation follow one another, whether upon the same individual, or on different individuals. An exact knowledge of the conditions gives the experimenter the secure control over the organism, which can at will be forced into any desired mode of propagation within the limits of its species. The problem becomes

more complex if other additional modes of propagation appear, as they do in many Fungi. In *Saprolegnia* we can distinguish four kinds of propagation: (1) by simple mycelial growth, that is, by breaking of the mycelium into pieces; (2) by zoospores; (3) by oospores; (4) by gemmae. The conditions for each of these four kinds of propagation are somewhat different, and it is thus possible, as my later investigations prove, to force the fungus at will into one or other of the four modes of propagation.

The potentialities for the several kinds of propagation in the Thallophytes, such as *Vaucheria*, *Oedogonium*, *Chlamydomonas*, *Sporodinia*, *Saprolegnia*, *Eurotium*, &c., are quite equivalent, i.e. there is no cause in the inner nature of the cell, or in the special organization of the potentiality, for one of these of its own initiative being developed earlier or later; nevertheless, according to the species, the special conditions which dominate the kinds of propagation may be more or less readily realized in open nature, as well as in the laboratory. In particular, the sexual propagation is often dependent upon more complex conditions than the non-sexual. While both can be induced without difficulty in *Sporodinia*, in other Mucorini it is most difficult to see the zygospores at all. I have not succeeded in my attempts to induce the formation of zygospores in the common and easily cultivated *Mucor racemosus*, though such formation doubtless exists.

In the works of Brefeld the idea is often expressed and tested of bringing a Fungus by culture through the most numerous successive conidium-forming generations to its higher fruit-form. This idea is connected with Brefeld's idea that inner causes are more important than external causes for the appearance of the fruit-form. The result of the serial cultures of Brefeld, whether positive or negative, was under all circumstances accidental. The experiments would prove the view of Brefeld only if the external conditions had really been always the same in all the numerous serial cultures. Since Brefeld, to judge from the meagre statements of his methods of culture, has not paid any attention to this constancy of the conditions, it will also have been a matter of chance whether they remained the same, or varied in such a way that another form of fruit took the place of that which preceded it. In any case, I may assert that if in Fungi such as *Sporodinia*, *Saprolegnia*, *Ascoidea*, *Eurotium*, those external conditions are maintained constantly which are characteristic for one of the forms of propagation, that same form only is produced. Hitherto

a vegetative growth of whatever duration, or a continued propagation in one form, has never of inner necessity led to the appearance of another.

We can now say that the majority of Algae and Fungi will behave like the species which have thus far been tested; in which behaviour the relation of dependence of the propagation, on each occasion, upon the outer world will vary extremely according to the species. If one recognizes thus far as operative factors light, temperature, moisture, oxygen, chemical composition of the nutritive medium, here is already at hand a great wealth of most various combinations of external stimuli which set the formative processes in motion. Further investigation will teach what a wealth of unexpected relations is here to be discovered between the outer world and organic life.

Despite all this, the possibility remains that in certain species a regular alternation of neutral and sexual generations does appear. That might be possible for the Florideae, in which the tetraspores and carpospores are often formed on special individuals. This simple fact proves nothing as yet, since it is faced by the other fact, that both kinds of propagation also appear from the like individual. The question remains open whether the tetraspores do not make their appearance at times, and seemingly on individuals other than do the carpospores, for the simple reason that the external conditions for the two of them are very dissimilar. The question cannot be decided till longer-continued cultures of the Florideae have been arranged. The answer will presumably not turn out differently from that in the case of other Algae. At the first glance an alternation of generations in Pringsheim's sense comes to much clearer expression in certain parasitic Fungi, especially in the Uredineae. If we leave aside the undecided question as to the occurrence of a sexual act, the observations and experiments teach that the life of a Fungus such as *Puccinia graminis* necessarily takes the course of the alternation of two independent generations living upon different host-plants, the one bearing teleutospores, the other forming aecidia. In addition there are still the subsidiary fruit-formations of the uredospores and of the spermogonia. In fact we have here a regular alternation of generations, such as appears in analogous form in the case of several of the lower animals; there is no obvious reason for avoiding the expression in this case, if one takes into account the actual circumstances of the case. But still it would be wrong to apprehend this alternation of

generations as if we had here an essentially new process, as against those other sorts of Fungi which are dimorphic or polymorphic. There are nearly allied Uredineae in which all the spore-forms appear one after another upon the same mycelium. In my view the condition for the different kinds of propagation will also be unlike, and the regular alternation of the fruit-forms would be explained by the fact that by the development of the host-plant itself, as by its dependence upon the seasons, changes in it necessarily go forward, which will serve as direct occasion for the growth of the different spore-formations of the parasite.

In *Uromyces Polygoni*, for instance, the aecidia appear only on the young plant, the uredospores and teleutospores on the older. In the heteroecismal Uredineae the special conditions for the individual fruit-formations are much more strongly distinguished still, so that other host-plants are necessary to bring the Fungus to the formation of aecidiospores or teleutospores. The time will probably come when these conditions may be more accurately recognized, and the Uredineae be cultivated on artificial substrata. Then it will appear whether these parasites do not behave just like the other Fungi, and cannot also be compelled to produce the different fruit-formations upon the same mycelium. A great obstacle to the cultivation of the Uredineae lies in our ignorance of the chemical composition of the host-plants. We are quite ignorant of the substances characteristic for the species, which, besides the usual food-stuffs, sugar, proteid, &c., are at any rate of decided importance for the development of the parasite.

In all the cases now mentioned we have to do with the alternation of several generations, each of which is characterized by special propagation. In the unicellular Thallophytes the non-sexual propagation coincides with the vegetative division. The propagation of the Desmidiaceae and Diatomeae by division corresponds to the propagation of *Chlamydomonas* by means of motile cells. In all of them the sexual process ensues after a series of divisions. Naegeli includes these processes under his conception of alternation of generations, and even extends it to the Bacteria in which, after a series of generations by division, the cycle is closed by the formation of endospores. But if the term alternation of generations be limited to organisms with dimorphic propagation, an alternation of shoots might be spoken of in this case.

Under all circumstances, whatever name the thing may bear, we must ask ourselves the same question as regards these phenomena as in the dimorphic Thallophytes; we must inquire whether a more or less definite number of cell-generations must be passed through before the fruit-generation can follow. In the Bacillus of anthrax (*Bacillus anthracis*), Buchner (1890) has already proved that it can be propagated as long as you please by division, and that at any moment the formation of spores can be induced by direct influence of the outer world. Schreiber (1896), who has closely investigated the conditions of spore-formation in several Bacteria, has been able to prove still more definitely that the spore-formation always begins as a direct consequence only of external conditions. Starting from the germinating spore, it was possible, after the third division, to induce spore-formation again. In *Chlamydomonas*, I was able to prove with certainty that the cells, through innumerable generations, propagated in an exclusively vegetative manner, but that at any time the formation of sexual swarm-spores can be attained with ease and certainty. Most probably the same holds for the Desmidiaceae, in which certain species may be propagated for many weeks together by division, but from the first were capable of sexual propagation when exposed to the conditions characteristic for it.

On the other hand, the Diatomeae seem to have a necessary alternation of generations, just as, according to the investigations of Maupas, the Ciliatae among the Infusoria. According to the theory founded by Pfitzer, the cells of the Diatomeae, whose silicified cell-wall consists of two parts fitted one within the other, do not grow in the direction which is usually styled longitudinal. The consequence of this is that on each division one of the daughter-cells maintains the length of the mother-cell, the other will necessarily be smaller by the thickness of the membrane. Thus by continued divisions the cells become smaller and smaller till, on reaching a certain minimum size, the process of auxospore-formation appears, by which the original maximum length is again attained. This generally acknowledged theory has been supported by the investigations of Miquel. He cultivated a number of Diatoms in artificial nutritive media, and noted in the successive generations a gradual lessening of the cells, till finally the formation of auxospores followed very freely. Thus, according to the statements of Pfitzer, Miquel, and others, the view might appear to be sufficiently established, that in

the Diatomeae the formation of auxospores follows only as a consequence of that organization of the cell which has been described after a number of divisions which may be almost mathematically defined, while the external conditions play no definite part in the process. But, meanwhile, we ought not to forget that this theory is in much need of further confirmation. The main point in the whole question is whether the cell-wall of both cells after division really does undergo no increase by growth in length to even a very small degree. Pfitzer himself has noted that such growth does occur in certain species, though this would be of no account for making up the loss of size which accompanies division. It ought to be distinctly proved, by direct and exact measurements, whether a growth in length takes place or not; above all, we ought to know precisely what influence the various external conditions exert upon the life of the Diatomeae. It may possibly be that under certain circumstances growth takes place, under others not. The fact, brought forward by Miquel and others, that it is by no means always the smallest cells which form auxospores, but also those of middle size, deserves consideration. Still more important are the statements made by Karsten, that *Melosira nummuloides* can be brought to the formation of auxospores simply by change of water, that in *Achnanthes longipes* the impending conjugation does not take place, but is replaced by vegetative growth when the cells are exposed to a cool temperature. If we assume that definite external conditions induce growth in length during division, others encourage auxospore-formation, the earlier observations on the smallness of the cells which form auxospores may thus be explained. In many Thallophytes the rule holds that in the stage of preparation for the higher fruit-form growth diminishes, or ceases, but division is still continued, so that, for instance, in the Desmidiaceae, *Spirogyra*, and *Chlamydomonas*, it is always the smallest cells which conjugate. This may also be the case on the formation of the auxospores of the Diatomeae, and the smallness of the cells would then be less the cause of auxospore-formation than the result of those external conditions which occasion this process. I bring this possibility forward in order to draw attention to the pressing need for accurately defining the conditions of the events in the life of the Diatomeae by the help of pure cultures, and by the use of physiological methods. Whichever way the decision may fall, the life-history of the Diatomeae gives no explanation of the wholly different alternation of generations

of the Archegoniatae, any more than does that of the other Thallophytes which have been mentioned.

But now the question arises whether there is not in many Thallophytes another form of alternation of generations, which presents nearer relations to the phenomena in the Archegoniatae. The fertilized ovum develops according to the statements of investigators in a definite way in certain species: thus, for instance, the zygospore of one of the Mucorineae, the oospore of *Vaucheria* and of *Saprolegnia*, usually germinate by formation of a short tube, which directly bears a sporangium. Pringsheim speaks, in such a case, of the first neutral generation: we might regard this as the actual spore-forming generation, corresponding to the sporophyte of the Mosses. Closer investigation shows that an oospore of *Vaucheria* shows no tendency in any way fixed by heredity to form a sporangium. It produces first a short germinal tube, which may either continue its vegetative growth, or may at once form zoospores, or sometimes sexual organs. That would depend alone on external conditions. De Bary and I myself have lately proved the same for the oospores of *Saprolegnia*, and Van Tieghem for the zygospores of the Mucorini. There is no true meaning in speaking of an alternation of generations in these cases, since the formation of the sporangia is not a peculiarity of germination, but follows the same conditions as it does subsequently. These plants do not behave differently in principle from the Fucaceae, or Conjugatae, in which the fertilized ovum passes more or less directly into the thallus, since no other propagation exists at all.

But there are perhaps other species in which the mode of germination of the oospores has become more definite. The zygotes of *Hydrodictyon* show, according to Pringsheim, a characteristic mode of germination, but it is not yet known exactly how far it is a constant process. In *Oedogonium* the germinating oospore forms four zoospores —a process which does not occur again in its later life. The manner of germination is not absolutely indispensable, since the oospore also passes over directly into a filament, though it appears to be the commoner case. Still more peculiar is the germination of *Coleochaete* as described by Pringsheim, in which the fertilized ovum divides and forms a tissue, from the cells of which zoospores arise, which then grow on into the typical thallus. There are no exact investigations whether this kind of germination is a constant phenomenon, since the germination has only been observed by Pringsheim, under conditions which were

apparently not very favourable. The formation of these bodies, which are like the zoospores formed elsewhere, from the tissue of the oospore, is perhaps a quite fortuitous circumstance, which differs in no way from the usual propagation. But the chief reason for comparison with the Mosses, and for the assumption of an alternation of generations, lies in the production of specially formed propagative cells from the oospore. It might be quite possible that the cells of the oospore in *Coleochaete scutata* should pass over directly into the thallus, but in *C. pulvinata* perhaps only after a change in the mode of growth. The essentially regular germination by help of a pro-embryo, as in *Chara*, will not be accepted as a formation of a special non-sexual generation. It is true that Vines has advanced such a conception, and compares the pro-embryo of *Chara* with the whole sporogonium of the Mosses. His view has not been taken up, since as a matter of fact in *Chara* a comparison seems permissible only with the protonema of the Mosses, or with the pro-embryo of *Batrachospermum*.

Next to *Coleochaete* it is the Ascomycetes and Florideae, the life-history of which has since the time of Sachs been compared with the alternation of generations in the Mosses, for in both the fertilized ovum has often a complicated development of its own; the last object of this is the formation of spores, which are clearly different from the usual propagative cells. As described by Schmitz, and according to the latest observations of Oltmanns (1898), the nature and method whereby the formation of the fruit depends on an intimate union of the fertilized egg-cell with definite auxiliary cells of the mother-plant is extremely peculiar. In the higher differentiated forms, e. g. *Callithamnion*, according to Oltmanns, a cell-derivative from the fertilized egg-cell—a cell-nucleus with some protoplasm—is united with a large auxiliary cell, and coalesces with it into a new cell-unit, whereupon the nucleus of the auxiliary cell is pushed aside as apparently functionless. This new cell, of which the wall and of which the plasma belong for the most part to the mother-plant, is stimulated by the 'egg-energid' to form the spores. In still more highly developed forms the mother-plant provides also for the enveloping of the spore-producing cells. Oltmanns compares the relation of egg-cell and mother-plant with that of a parasite and its host-plant, and sees therein a confirmation of the view that the fruit of the Florideae corresponds to the sporogonium of the Mosses. But

one may also designate the state of the facts with this expression: that the fruit of the higher Florideae is a product of the mother-plant that is stimulated by the fertilized egg-cell. Pringsheim, at least, might, in the far-reaching dependence of the fruit-formation upon the mother-plant, find a substantial support for his view, that the fruit of the Florideae has not the value of a special non-sexual generation. Finally we have to consider subjunctive interpretations. I myself should hold the comparison of the Floridean fruit with the sporophytes of the Mosses as quite justified. But one essential point in this matter ought not to be forgotten. One may compare the fruit of the simple Florideae with that of the simple Liverworts, and apprehend both as in some degree analogous structures. But a very important and interesting difference discloses itself, if one follows up the line of development in the two series. In the Mosses the effort is distinctly marked in the ascending series of forms to differentiate more highly the sporogonium as an immediate product of the fertilized ovum, and to make it more independent of the mother-plant in its nourishment. In the series of the Florideae the opposite tendency shows itself to make the development of the fertilized ovum constantly more strongly dependent on the mother-plant, and to attain the higher differentiation of the fruit by means of essential co-operation of the mother-plant. Beyond this no one will wish to assert a nearer relation of kinship between Mosses and Florideae.

It is still more difficult in the Ascomycetes to decide the question of the alternation of generations than in the Florideae. Notwithstanding the remarkable differences which are to be observed in them up to the origin of the ascus-fruit, we must still, with De Bary, regard the whole group as a single and united one. But we ought not to connect the Ascomycetes with the Phycomycetes, either with the Peronosporeae after De Bary, or with the Mucorineae after Brefeld; but we should recognize in them a group which, with its simplest forms, sends out its roots into the lowest division, the Archimycetes, to which the Chytrideae and other Fungi belong. In quite simple Ascomycetes, e. g. *Ascoidea*, *Dipodascus*, *Endomyces*, there appears a striking difference in the mode of origin of the asci. In *Ascoidea* and *Endomyces*, according to Brefeld, each ascus arises directly from a mother-cell of the mycelium. In the nearly-related *Dipodascus*, according to Lagerheim, two cells coalesce, and it is the product which grows on into the ascus. In the one case—apparently the more common one—the

ascus is a direct product of the mother-plant; in the other forms we may speak of the beginning of a non-sexual generation. If we pass on to the fruit-bearing forms in some still relatively simple species, we find the asci as products of a fertilized ovum, e. g. in *Sphaerotheca* according to Harper, or the Laboulbeniaceae according to Thaxter. In others we may regard a structure homologous with the ovum, but not actually capable of fertilization, as the starting-point for the formation of asci, while other constituent parts of the fruit, such as the wall, and commonly the stalk, &c., are supplied by the mother-plant. In the highest forms the most complicated Pyrenomycetes, and the *Cladonias* among the Lichens, &c., the fruit is, according to our present knowledge, exclusively a product of the mother-mycelium, just as is the case according to Brefeld in the Basidiomycetes. It is only in case of the simpler forms that we can compare the ascus-fruit with the sporogonium of the Mosses, and, as Oltmanns has done, place it in relation to the processes in the Florideae. In the Ascomycetes it is still more clearly to be recognized than in these plants that the antithetic alternation of generations has stood still at the first attempts, and in the higher forms has been replaced by direct development of the fruit from the mycelium. As regards the solution of the question how the alternation of generations in the Archegoniatae came into existence, the Ascomycetes can contribute far less than the Florideae.

Taking a general view of the department of the Thallophytes thus traversed, the following cases may be distinguished as relating to the question of the appearance of an alternation of generations:—

1. The majority of the Algae and Fungi have two or more kinds of propagation, each of which necessarily depends upon definite external conditions characteristic for it. According to the conditions, occurring fortuitously in open nature or in cultivation, the kinds of propagation may appear on the same or on different individuals, independently or in any succession. The fertilized ovum in sexual forms does not differ essentially on germination from another propagative cell. In none of these cases is there any reason for speaking of an alternation of generations.

2. In certain heteroecismal parasites, e. g. many Uredineae, the life-history of the species takes the course of a regular succession of different individuals with special modes of propagation; we may here speak of an alternation of several generations characterized by different

propagation. There would be in this case an alternation of homologous generations in the sense of Celakovsky and Bower. Here also we have essentially to deal only with Fungi which have dimorphic or polymorphic propagation, with the limitation that the external conditions for some of the forms of propagation are so different that, so far as experience yet goes, these are only developed upon separate host-plants.

3. In the unicellular Diatoms there is, according to the theory of the cause of auxospore-formation hitherto current, an alternation of generations in the sense that, after a definite number of cell-generations derived by division, the formation of auxospores follows by internal necessity. But it needs still more exact investigation, for it is quite possible that the formation of auxospores, like the formation of zygotes of the Desmidiaceae, is essentially dependent upon external conditions. In that case there would be no definite alternation of generations in the Diatomeae.

4. In a number of Thallophytes, some few Chlorophyceae, above all in the Florideae and Ascomycetes, a fruit arises from the fertilized ovum, or a body homologous with it, which produces, in a manner peculiar to it, non-sexual cells, the spores. This spore-bearing fruit may be compared with the sporogonium of the Mosses, and the alternation of the sexual plant with the spore-fruit may be regarded as an antithetic alternation. But this comparison does not extend further than the establishment of a certain analogy.

In the two series of the Florideae and Ascomycetes, in contrast to the series of the Mosses, it appears that the fruit in the higher forms becomes constantly more dependent upon the mother-plant, and that the duty of higher differentiation of the fruit falls essentially upon the latter. The fruit there appears not as a special generation, but as a product of the mother-plant.

What, then, remains from which to derive the alternation of generations of the Mosses and Ferns? Only *Coleochaete*, which, since Pringsheim's celebrated investigation, has been quoted as a connecting link between Algae and Archegoniatae. But it has never been proved that the zoospores of the germinating oospore are to be regarded as a characteristic product of the fruit, and, accordingly, as a form of propagation homologous with the Moss-spores. But, on the other hand, it makes no great demand on our imagination to figure to ourselves how, in the *Coleochaete*-like ancestors of the Mosses, this step

was taken, that then the second step consisted in the formation of stationary spores arising by a tetrad division. With such assumptions, the transition to the simple Liverworts—e.g. *Riccia*, does not appear very great, and, starting from this form, the different series included in the Bryophyta may be derived. Though we have thus gained certain connecting points for the phylogeny of the Mosses, the question as regards the Ferns, in which the fertilized ovum develops into the leafy plant, is in quite another position. It has been recognized on many sides how great a contrast there is between Mosses and Ferns. The common peculiarity in the structure of the archegonium might be a purely parallel development without its necessarily indicating any phylogenetic connexion. It is not my purpose to enter now upon these difficult questions, the less so since they will be dealt with here from an official quarter. They deal with the most interesting, but also the most obscure, points in the phylogeny of the vegetable kingdom. For the spot where the first indication of a Fern-sporophyte appeared was the birthplace of the vastly-developed series of the Phanerogams. The Thallophytes hitherto known do not give the least clue to the discovery of that spot.

GEORGE KLEBS, Tübingen.

ALTERNATION OF GENERATIONS IN THE ARCHEGONIATAE.—One of the most important facts in the morphology of all plants higher than Thallophytes is the occurrence in their life-history of two alternating stages, which differ widely from each other both in structure and reproduction. Of recent years advances in our knowledge in several distinct departments of botanical investigation have raised anew the question of the nature of this Alternation of Generations. The subject has been discussed from two very different standpoints in the Presidential Addresses to this Section of the British Association this year and at the Liverpool Meeting[1]. These expressions of opinion by Dr. Scott and Professor Bower render an introductory paper to this discussion in one sense superfluous. While, however, repetition of much that has been already said is unavoidable, the existence of such diverse views suggests a slightly different treatment of the question, which may be useful for the

[1] The existence of these recent statements of the problem renders references to the literature of the subject unnecessary.

purposes of the discussion. Instead of advocating either the theory of antithetic or of homologous alternation, I shall try to present a dissection of the subject; with this object the main facts known as to alternation of generations will be briefly discussed, and the possible interpretations of them considered. The facts will as far as possible be kept apart from the theoretical views to which they have given rise, and the points on which our knowledge is deficient will be emphasized rather than minimized.

The general facts regarding alternation of generations in archegoniate plants can be dismissed very briefly. In all the main groups a definite alternation of a sexual with an asexual generation is found. The latter is normally developed from the fertilized ovum, the former from the spore. The Bryophyta and Pteridophyta are, however, opposed to one another in the relative complexity attained by the two generations. The sporophyte in the Bryophyta remains dependent on the Moss or Liverwort plant, and has as its main function the production of the spores. It may, however, attain very considerable complexity of structure and possess a well developed assimilation tissue. In both Hepaticae and Muscineae very simple sporogonia lead on to complex ones in which the sterile tissue of the wall, foot, seta, &c., forms a considerable proportion of the whole structure. The gametophyte, on the other hand, is always independent and often shows a complicated external form with clearly differentiated stem and leaves. In the Vascular Cryptogams also the gametophyte is always independent, but is of relatively simple form and structure. The sporophyte, which develops from the fertilized ovum, very soon produces roots, and attains independence by the death of the prothallus. It shows a distinction of stem and leaf, is highly organized, and does not develop spores until after a period of vegetative growth. While these points of difference which indicate the great gap between the Bryophyta and Pteridophyta are borne in mind, due weight must be given to the points of agreement. Of these, the similar structure of the sexual organs, the fact that in both the sporophyte is at first dependent on the gametophyte, the presence of stomata and intercellular spaces in the sporophyte, and the similarity in the spore-production may be mentioned. A consideration of these facts by themselves indicates no view as to the mode of origin of the two generations. At no stage do the two generations in any Archegoniate closely resemble one another, except in the case of the young plant

and the prothallus of *Lycopodium cernuum*. The deviations from the normal life-history, which will be considered later, may somewhat modify this statement.

We are justified in assuming that the Bryophyta and Pteridophyta arose from ancient Thallophytes; the study of the life-histories of the Algae and Fungi, which exist at present, may accordingly be expected to aid in arriving at probable conclusions as to the origin of the alternation in archegoniate plants. It is naturally among the Green Algae that indications of this sort might be expected, nor are they wanting, though the precise weight to be attached to them is a matter of uncertainty. The higher Fungi and the Red and Brown Algae may for the sake of simplicity be left on one side with the remark that in Ascomycetes and Florideae we see a development which presents analogies with the alternation in Archegoniates. Confining ourselves to the Green Algae and the simpler Fungi we find among them two sorts of phenomena which have been termed alternation of generations. Most of these organisms reproduce both sexually and asexually, and sexual and asexual individuals, resembling one another in their vegetative structure, are often found. The same individual may, however, bear both kinds of reproductive organs, and Professor Klebs has shown in a number of cases that the mode of reproduction is largely determined by the external conditions and can be brought under experimental control. There is thus no doubt that these sexual and asexual individuals are homologous in the full sense of the term. But there are a number of Thallophytes in which another stage in the life-history is found, which, by its regular recurrence and the position it occupies in the life-cycle, suggests a comparison with the sporophyte of the simpler archegoniate plants. While in many Thallophytes the fertilized ovum or the zygospore develops directly into an independent plant resembling the parent, in these it first divides into a number of cells, which are usually motile spores, but may form a small mass of tissue from the cells of which swarm-spores arise. It is sufficient to mention *Oedogonium*, *Cystopus* and *Coleochaete* as organisms which show this clearly. In the life-history of *Sphaeroplea* only sexual individuals and the group of swarm-spores, which results from division of the oospore, alternate, independent asexual individuals not being found.

If we now consider how this second form of alternation in Thallophytes might have come about, without for the moment extending

our view to archegoniate plants, the essential distinction of the antithetic and homologous theories will become plain. Further, we shall here be dealing with a problem with regard to which the work of Professor Klebs justifies the anticipation that direct evidence will sooner or later be obtained. The main question at issue is, In what relation does the group of spores in *Oedogonium*, or the small mass of tissue resulting from the division of the fertilized ovum in *Coleochaete*, stand to the asexual individuals of the same species? There is some evidence that in this stage we see the representative of an asexual individual, the vegetative body of which has become more or less completely reduced. Thus occasionally in *Oedogonium* a vegetative individual develops from the zygote; in *Uothrix* the zygospore develops a rhizoid, but the contents of what appears to be a rudimentary plant are wholly devoted to the formation of motile spores. On this view the cell-mass in *Coleochaete* would be regarded as a reduced thallus, all the cells of which form spores asexually. The reduced generation which proceeds from the zygote would genealogically correspond to an independent asexual individual, and just as the latter is homologous with a sexual individual, so would the four spores in *Oedogonium* or the cell-mass in *Coleochaete* be. This would be homologous alternation of generations.

But the same facts can be viewed in another light. In all these cases the advantage to the plant in producing almost at once a number of individuals instead of one as the result of the sexual act is obvious. The division of the ovum may have originated as a special adaptation to this end, and not represent a reduced first neutral generation at all. In the life-history of these plants there would then be a stage not represented in the majority of the Thallophytes, which may in this sense be spoken of as interpolated. The cell-mass of *Coleochaete* upon this view would not represent a less reduced neutral generation, but a more complicated development of the interpolated stage, which is seen in its simplest form in *Oedogonium*. This stage would not correspond to, or be homologous with, the independent asexual individuals, and leaving these out of account, only one individual, and the result of elaboration of its zygote would be represented in the life-history. The alternation would not be homologous but antithetic.

If we now proceed to apply these two points of view to the facts of alternation in the Archegoniatae, the problem in its most general

form is this: Is the sporophyte in the Bryophytes and the Vascular Cryptogams to be ultimately traced back to modification of a genealogical individual homologous with the gametophyte, or is it the result of still further elaboration of an interpolated stage more or less like that seen in *Coleochaete*? On the antithetic theory the sporophyte is traced increasing in complexity through a series of forms illustrated by *Oedogonium*, *Coleochaete*, *Riccia*, *Marchantia*, *Anthoceros*, and the simplest sporophytes of the Vascular Cryptogams are regarded as having been derived from a sporogonium, which already possessed a considerable amount of sterile tissue. If, on the other hand, we apply the homologous theory, several alternatives present themselves. The first, which is not widely different from the antithetic theory, is that in the course of its descent the sporophyte of the Vascular Cryptogams has passed through a stage resembling the Bryophyte sporogonium, but that the origin of this second generation in the ancestral Algae was homologous. But the homologous theory does not necessarily assume the existence of the sporogonial stage. The sporophyte of the Vascular Cryptogams may have had an independent origin from that of the Bryophyta, and have resulted from the modification of individuals, which were never reduced to the condition of a fruit body.

As to the circumstances which led to alternation of generations, the two theories are in essential agreement. We owe to Professor Bower the general statement, which must serve as the starting-point of any explanation, that the origin of the alternation may be correlated with a change of habit from aquatic to sub-aerial life. This holds whether the second generation is considered to be homologous with the first, or to be the result of interpolation. On the latter view, which is that elaborated by Professor Bower, the importance of the drier conditions of life is sought in the prevention of repeated acts of fertilization. It would thus have been an advantage to the organism to produce many individuals as the result of one sexual act, and this is seen to be effected with increasing perfection as we pass from the simpler to the more complex Bryophyte sporogonia, and from these to the Pteridophyta. The same change of environment may, however, have initiated the modification of individuals, which were originally potential sexual plants, into spore-bearing forms. We shall return to this when discussing apogamy.

We have seen that the facts of morphology do not of themselves

indicate decisively which theory is the correct one. The reasons which render one or the other view the more probable are bound up with the more general question of the course of descent in the vegetable kingdom. The question of the relationship between the main groups of plants is a very complex one. All that we need do here, however, is to recognize the existence of several alternative views, and the bearing of these on the two theories of alternation. The indications of alternation in the Thallophytes may be first referred to. These seem closely comparable to the simplest Liverwort sporogonia, but it has not been suggested that any direct relationship exists in any case. The existence of these rudimentary sporophytes in various Green Algae, in *Cystopus*, and in an analogous, though distinct form in Ascomycetes and Florideae, is indeed strongly suggestive of their independent origin in the Thallophytes of the present day, and justifies us in considering it probable that similar developments may have occurred in the ancestral Algal forms from which the Archegoniates arose. But the further recognition of the possibility that the origin of the Archegoniatae may have been polyphyletic, and in particular that the Vascular Cryptogams may have had a line of descent from Thallophytes perfectly distinct from that of the Bryophyta, has a much more important bearing on the nature of alternation. The gap between Bryophytes and Pteridophytes is wide, and on this view would be an essentially natural one; any attempt to bridge it would involve misleading conclusions. I do not wish to enter into the question of the polyphyletic origin of archegoniate plants further than to show that its possibility must be borne in mind in considering the nature of alternation. It may be pointed out, however, that such a view would appear to follow naturally from the supposition that the origin of the sporophyte was correlated with the spread of aquatic organisms to the land. It may be considered probable that a number of organisms in different places would have undergone more or less similar modifications. The homologies which exist between the spore-bearing generations of Mosses and Ferns are no less possible results of homoplastic developments than others in favour of which direct evidence exists. If the origin of the Pteridophyta has not been from the Bryophyta, the comparison between the sporogonia of the latter and the simpler sporophytes of the Vascular Cryptogams would lose much of its weight, since the two may have proceeded, as Goebel

suggested, on distinct lines from the beginning. It is therefore advisable to ascertain if any evidence exists which may indicate how the Vascular Cryptogams could have been derived directly from Algal forms. Something of the kind, as we shall see, may possibly be afforded by the facts of apogamy.

So far we have seen no reason to regard the nature of alternation and the views on descent which underlie it as anything but open questions. There are, however, two important classes of facts, which have been regarded as affording more direct evidence in favour of the antithetic and homologous theories respectively. These are the cytological differences between the two generations, and the deviations from the normal life-history known as apospory and apogamy.

The first of these will only be mentioned. The existence of the double number of chromosomes, which results from the sexual fusion, in the nuclei of the sporophyte, throughout Bryophyta, Ferns, and the higher plants, certainly appears to lend support to the view that the sporophyte is an interpolated stage in the life-history. From the cytological point of view the intercalation is between the doubling of the number of chromosomes by the sexual fusion and the reduction in number in the spore mother-cells. Facts are wanting as to the nuclear changes in Thallophytes, and also in apogamy and apospory.

These latter phenomena are the last element in the problem that can be referred to at length. We saw that in the case of the alternation of clearly homologous generations in the Thallophyta it had been shown that the assumption of the sexual or asexual form depends on the external conditions. This experimental study needs to be extended to the rudimentary sporophytes of the Green Algae, but with regard to these it is already known that in *Oedogonium* the fertilized ovum may grow out directly into a vegetative plant, instead of dividing into spores. In the Archegoniatae this complete substitution of one generation for another is not known to occur; no variations in the external conditions are known to induce a Fern-spore to develop into a Fern-plant, or the fertilized ovum to give rise directly to a prothallus. But the facts as to the direct development of the one generation from the tissues of the other, and the existence of structures which may fairly be described as intermediate between gametophyte and sporophyte are sufficiently striking.

The main facts with regard to apospory, the vegetative origin of the

gametophyte from the tissues of the sporophyte, are briefly these. In Mosses cut portions of the seta or capsule have been induced to give rise to protonemal filaments; in one case this is known to have occurred in nature while the capsule was still attached to the Moss plant. In a number of Ferns the production of prothalli from the sporangia, the placenta, the surface of the leaf or the leaf margin, takes place. In *Scolopendrium vulgare* and *Nephrodium Filix-mas* varieties are known in which the first leaves of the young sporophyte exhibit this capability of producing prothalli. The causation of this phenomenon is still obscure. In a number of cases sporal arrest has been shown with probability to be of importance, notably in the case of *Onoclea*, in which apospory occurred on fertile leaves which had been experimentally induced to assume the vegetative form. Further, the fact that conditions of life favourable to the gametophyte, such as laying the fronds on damp soil, determine the growth of prothalli from the tissues of some aposporous Ferns may be mentioned. As to the weight to be attached to apospory it must be borne in mind that the phenomenon is little more wonderful than the fact of the spore, a cell isolated from the sporophyte, producing a prothallus. Here, as in the case of apogamy, the investigation of the cytological details is urgently needed.

The deviations from the normal life-history, which are classed as apogamy, may be considered to possess more importance as suggesting the homology of the two generations in the Ferns. Though as yet only known in this group of plants, apogamy has been found in more than twenty species. In some the young Fern-plant arises on the under surface of the prothallus, which in these cases often bears few or no sexual organs. But in cases in which apogamy has been induced the characters of the two generations may be much more intimately blended. Thus tracheides may occur in a prothallus more or less modified in external form. This may grow on as a bud, or may bear isolated members of the sporophyte, leaves, roots, ramenta, or sporangia. The characters of the two generations are here united in the same individual in a way that at least suggests a gradual transition from gametophyte to sporophyte.

It is to be hoped that the further study of these deviations from the normal development will lead to their causation being made clear. This may minimize the importance to be attached to them, especially should they be found to depend on a nuclear change. The facts

regarding the cytology of these new growths are still unknown; it is not even certain that the cells of the aposporously produced prothalli possess the half number of chromosomes, and those of the apogamously produced sporophytes the double number, though this may be assumed to be probable. Apospory at least might be readily explainable by such a nuclear change.

With regard to apogamy, however, some general conclusions may fairly be drawn even in the absence of observations on the nuclei. For whatever change may take place in the latter, it is certain that the transition from prothallus to sporophyte, or from prothalloid to sporophytic tissue, takes place without relation to the sexual fusion, and is so far comparable to an ordinary variation. Further, it is to be noted that the change takes place, so far as the conditions are known, when, by preventing the access of fluid water, fertilization is delayed, and when in other ways the conditions approach those favourable to the sporophyte rather than the gametophyte. These modifications of the conditions are of the kind to which aquatic organisms would be exposed on assuming a terrestrial habit. It is, therefore, possible to view the changes which take place in prothalli under these circumstances, not as reversions, but as indications of the capability of the gametophyte to assume the characters of the sporophyte under suitable conditions. If there is any truth in this way of regarding the facts of apogamy, they become of value in enabling us to picture the steps by which the Fern-sporophyte may have arisen by changes in individuals homologous with the original sexual form. The prothallus, especially in the Ferns, must have departed much less widely from the ancestral Algal form than the sporophyte; this may be connected partly with the conditions to which it remains adapted, and partly with the fact of its growth being in nature cut short by the early formation of the embryo upon it. The various cases of apogamy which have been observed form an almost complete series of transitions between prothallus and sporophyte, and have been used to frame a provisional hypothesis of how the alternation in the Ferns might have arisen, if it did not come about in the way suggested by the antithetic theory.

All such use of the facts of apogamy and apospory is liable to the criticism that they are teratological in their nature, and are not a safe guide in a morphological question of this sort. There are many facts which go far to justify such a view, but we should, I venture to think,

be unwise to leave the consideration of these phenomena altogether on one side. Not only can no sharp line be drawn between variations (the use of which in evolutionary questions none will deny) and monstrosities, but, apart from the particular organic forms which result, we appear to be dealing with a capability of many—perhaps all—Fern-prothalli to assume characters of the sporophyte; a general property of the gametophyte of this kind cannot be disregarded A fuller knowledge than we possess of the causes of apogamy is, however, necessary before the bearing of the phenomena on the nature of alternation can be properly estimated; such knowledge may lead to an explanation more in accordance with the antithetic theory than any which has yet been given.

Whether the homologous or the antithetic theory is to be considered the more probable has an obvious bearing on morphology. But there is a wide difference between considering the two generations homologous with one another in the sense that the spore-bearing generation is ultimately to be traced back to modification of the sexual, and the view that any special structure of the sporophyte is strictly homologous by descent with any structure in the gametophyte. Special evidence would be necessary before such a conclusion could be drawn, and, so far as I am aware, no such case has been shown to exist. Not only, then, does the question of the nature of alternation of generations in the Archegoniates appear to be an open one, but there seems no reason to apprehend confusion in comparative morphology, whichever of the two theories be adopted as a working hypothesis.

In concluding this account of some of the main factors in the problem which is the subject of this discussion, three subsidiary questions may be suggested—the probable line of descent in archegoniate plants, the bearing of the cytological facts on the question, and the significance to be attached to apospory and apogamy. None of these questions, any more than the general one of the nature of alternation, may admit of a decided answer. It can, however, hardly fail to be productive of good if this discussion enables us to see our way more clearly to the directions in which the answers to these problems must be sought.

W. H. LANG.

QUEEN MARGARET COLLEGE, GLASGOW.

ALTERNATION OF GENERATIONS.—The first thing to be considered in this question is the behaviour of the cells. We may distinguish two modes of cell-division: (1) the *protistoid*, where the division of the cells is not influenced by their forming part of a complex multi-cellular body, and (2) the *colonial* or *metistoid*, where the division of every cell is dependent on its relations to the other cells of the organism at large. In Metazoa and Fucaceae a cycle of metistoid cell-division, in the formation of the organism with its buds and branches, alternates with a single short cycle of protistoid cell-divisions which produce the gametes—the oospheres (or oosphere and polar bodies) on the one hand, and the spermatozoa on the other. In archegoniate plants, and less visibly in Flowering-plants, there are two such alternating cycles of protistoid and colonial cell-multiplication: (*a*) starting with the zygote, we have the colonial system of the sporophyte, followed by the protistoid divisions determining the formation of the neutral spores; (*b*) starting with the spores, we have the other colonial formation, that of the gametophyte, eventuating in the protistoid formation of the gametes. We have here a double alternation of twofold cycles, which thus cannot be paralleled by anything in the life-history of the lower Thallophytes, save in so far as they exhibit true tissue-formation and colonial subordination of cells. The alleged alternation in *Batrachospermum*, for instance, is really comparable with larval conditions. That we are justified in the comparison of the ill-developed sporophyte in the Muscineae with the stately 'plant' of the higher Archegoniates, is shown by the formation of such peculiar organs as true stomata in both; and that this is not a mere case of homoplasy is shown by the fact that the conditions under which stomata are needed are found in the Marchantiaceae, and that breathing-pores which are not homologous with true stomata are here found. Again the phenomenon of nuclear reduction occurs at the same stage in both groups, to which we shall refer later.

The argument put forward that the Moss-sporophyte must be considered 'part of' the leafy plant because it is attached to it and never becomes free, takes no cognizance of the discontinuity of the oosphere at the moment of its fertilization, and the lack of continuity of the cell-network of the sporogonium with the leafy plant; it disregards the necessary results of parasitism; and if applied to the Vertebrata, would lead to the most curious jumbling of questions of individuality in the passage from oviparity to viviparity, with the varying intensity of the parasitism of the embryo on the mother.

Why should there be such complex alternation in the archegoniate phylum alone? The Green Flagellates show an exceptional polymorphism among the Protista; and we may fairly ascribe this to the fact that by their holophytic nutrition, manufacturing their own foodstuffs, they can grow freely and multiply in the encysted state; while holozoic feeders can only do so when in the free condition. We find extreme cases of this polymorphism in the genus *Ulothrix*, where the forms are adapted for all sorts of substrata, differing in moisture and exposure; and the reproductive modes show a tendency to similar polymorphism. It is from such a stock (but thalloid, not confervoid) that we may well suppose the Green Metaphytes to have sprung. If here, owing to greater permanence of the thallus that bears the oosphere, the zygote or product of its fertilization has the opportunity of parasitism on the parent thallus, we can easily see the possibility that its resolution into a brood of spores might be delayed with the formation of a new colonial condition, at first parasitic on the mother as in Muscineae, with partial consequent sterilization of its tissues, and then an acquisition of independence, with the sterilization carried to the extent that we find in higher Archegoniates and Flowering-plants. As to nuclear reduction, we may attempt to ascertain its significance by classifying the stages at which it occurs. In Desmids, &c., it occurs at the first divisions of the zygote. These plants have no colonial tissue-formation. In Fucaceae and Metazoa, it occurs in the first gametogenic cell-divisions; these have the single alternation of colonial and protistoid cell-multiplication; in Archegoniata, and higher plants, it occurs at the inception of the sporogenic cell-divisions, the first resumption of protistoid cell-multiplication after the doubling of the number of chromosomes by fertilization. For the present I omit the case of the Ciliata, so exceptional from all other Protista in their cytology. We may group these cases in what the mathematicians would call an 'interpolation formula,' which covers our present knowledge and which would run thus: *Nuclear reduction is the reduction of the number of chromosomes due to the union of cells in fertilization to that found in a single gamete; it occurs in the* first protistoid divisions *that occur after fertilization; and is the necessary secondary result of fertilization, which would otherwise lead to the indefinite increase of the number of chromosomes by constant doubling.* This view is due partially to Strasburger, partly to the writer.

M. HARTOG.

QUEEN'S COLLEGE, CORK.